Food and Agriculture Organization of the United Nations

Guide to the classical biological control of insect pests in planted and natural forests

FAO FORESTRY PAPER

182

ISSN 0258-6150

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Guide to the classical biological control of insect pests in planted and natural forests

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182

by
Marc Kenis
Brett Hurley
Fernanda Colombari
Simon Lawson
Jianghua Sun
Carlos Wilcken
Ronald Weeks
Shiroma Sathyapala

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2019

Required citation:
Kenis, M.; Hurley, B.P.; Colombari, F.; Lawson, S.; Sun, J.; Wilcken, C.; Weeks, R. and Sathyapala, S. 2019. *Guide to the classical biological control of insect pests in planted and natural forests*, FAO Forestry Paper No. 182. Rome, FAO.
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ISBN 978-92-5-131335-0

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Cover photo: The sirex woodwasp, a serious pest of *Pinus* species. © B. HURLEY

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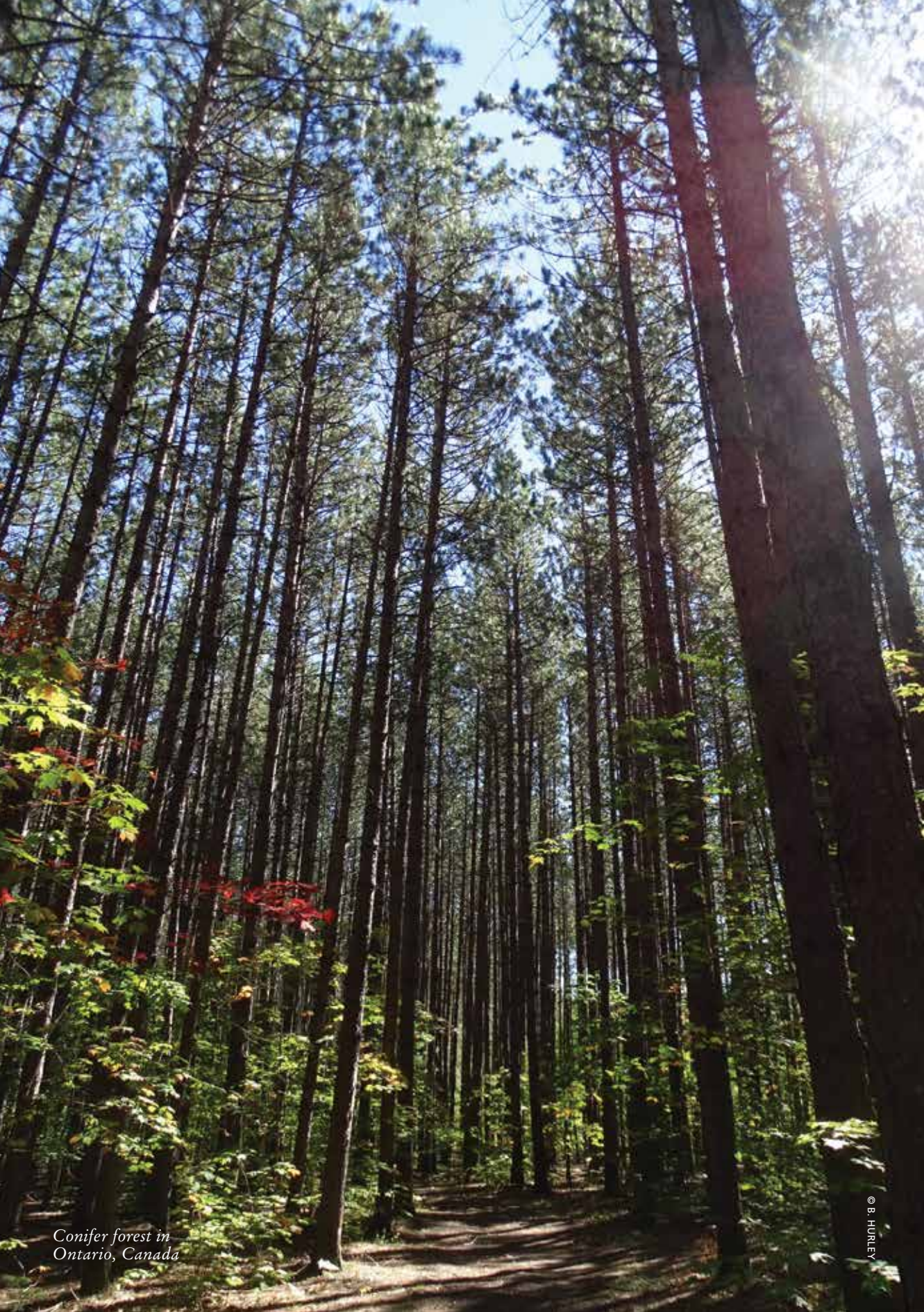

Conifer forest in Ontario, Canada

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Foreword

Insect pests damage millions of hectares of forest worldwide each year. Moreover, the extent of such damage is increasing as international trade grows, facilitating the spread of insect pests, and as the impacts of climate change become more evident. Globally, and especially in developing economies, outbreaks of forest pests can have major consequences for the livelihoods of forest-dependent communities.

FAO views the threat posed by forest insect pests very seriously. Pest management is an important element of Sustainable Development Goal 15 ("Life on Land"), especially target 15.8: "By 2020, introduce measures to prevent the introduction and significantly reduce the impact of invasive alien species on land and water ecosystems and control or eradicate the priority species". FAO's ongoing programme on integrated pest management supports the achievement of this target, including through classical biological control.

Classical biological control is a well-tried, cost-effective approach to the management of invasive pest species. It involves the importing of "natural enemies" (parasitoids, predators and pathogens) of non-native pests from their countries of origin with the aim of establishing permanent, self-sustaining populations capable of reducing pest populations to below acceptable damage thresholds. Classical biological control is especially well suited as a management tool for exotic pests that invade new environments, and it can provide long-term, efcacious control at minimal ongoing cost. It also poses risks and it is crucial, therefore, that it is implemented with adequate safeguards.

A great deal of knowledge on classical biological control has been accumulated worldwide in the last few decades. This publication, which was written by a team of experts, distils that information in a clear, concise guide aimed at helping forest-health practitioners and forest managers – especially in developing countries – to implement successful classical biological control programmes. It provides general theory and practical guidelines, explains the "why" and "how" of classical biological control in forestry, and addresses the potential risks associated with such programmes. It features 11 case studies of successful efforts to implement classical biological control around the world.

I thank everyone involved in producing this document, especially the authors and all those who participated in the expert meetings that gave rise to the work. I have no doubt that the application of this guide in diverse situations will help in the safe, effective use of classical biological control and thereby in ensuring the health and productivity of the world's forests.

Hiroto Mitsugi
Assistant Director-General
FAO Forestry Department

Acknowledgements

Shiroma Sathyapala, FAO Forestry Officer (Forest Health and Protection), coordinated the preparation of this publication under the overall guidance of Eva Müller, Director of Forestry Policy and Resources, FAO Forestry Department. Two international expert meetings were convened to assist in preparing the publication, the first in Rome, Italy, in March 2017 and the second in Langkawi, Malaysia, in September 2017. FAO thanks Carl Rankin for facilitating both meetings as well as online consultations and for preparing a first draft of the document based on inputs received from experts. The following members of a peer-review group are acknowledged for their contributions and comments on various drafts of the publication: independent reviewers Professor Timothy D. Paine and Dr Eston Mutitu; Avetik Nersisyan and Sandra Goritschnig from the International Plant Protection Convention; Andrei Orlinski from the European and Mediterranean Plant Protection Organization; and Mette Løyche Wilkie from the FAO Forestry Department. FAO acknowledges the contributions made to the needs-assessment survey by members of the Asia-Pacic Forest Invasive Species Network, the Forest Invasive Species Network for Europe and Central Asia, the Near East Forest Health and Invasive Species Network, the Forest Invasive Species Network for Africa, and Federica Urbani at FAO. Many thanks to technical editor Susan House, copyeditor Alastair Sarre and designer Kate Ferrucci for their contributions.

FAO also thanks the authors of this guide: Dr Marc Kenis, Centre for Agriculture and Biosciences International, Switzerland; Dr Brett Hurley, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; Dr Fernanda Colombari, University of Padova, Italy; Dr Simon Lawson, University of the Sunshine Coast, Australia; Professor Carlos Wilcken, São Paulo State University, Brazil; Dr Jianghua Sun, Institute of Zoology, Chinese Academy of Sciences, China; Dr Ronald D. Weeks, United States Department of Agriculture, United States of America; and Dr Shiroma Sathyapala, FAO, Rome.

Acronyms

BCA	biological control agent
BS	biosecurity
CBC	classical biological control
CBD	Convention on Biological Diversity
FAO	Food and Agriculture Organization of the United Nations
IOBC	International Organisation for Biological and Integrated Control
IPM	integrated pest management
IPPC	International Plant Protection Convention
ISPM	International Standard for Phytosanitary Measures
NPPO	national plant protection organization
PC	physical containment
PRA	pest risk analysis
WHO	World Health Organization

The parasitoid Apechthis compunctator *parasitizing a boxwood tree moth (*Cydalima perspectalis*) pupa*

© T. HAYE

Introduction

1.1 The threat to forests posed by insect pests

Planted and natural forests provide many economic, social and environmental benefits. For example, they help combat desertification, protect watersheds, regulate climate, conserve biodiversity and maintain social and cultural values. In economically developing countries, forests help meet basic needs for food, medicines, timber, energy and fodder.

Insect pest outbreaks damage about 35 million hectares of forests annually, mainly in the temperate and boreal zones (FAO, 2010). Such outbreaks are increasing globally as the volume and speed of international trade escalates and the climate changes.

In planted and natural forests, endemic insects and tree diseases are integral components of ecosystems. The introduction of non-native insect pests to new areas where they have no natural enemies, however, can have catastrophic impacts. Damage caused by invasive forest insect pests can reduce tree growth and timber quality and production, cause declines in biodiversity, significantly affect vital forest ecosystem functions and change entire landscapes. Although native forest pests can have similar effects, usually these are more sporadic and can be managed by modifications to management regimes.

In economically developing countries, non-native forest insect pests can cause severe economic losses, affecting the livelihoods and food security of many forest-dependent people. It is crucial, therefore, to manage insect pests appropriately. Pest management is an important element of Sustainable Development Goal 15 ("Life on Land"), especially target 15.8: "By 2020, introduce measures to prevent the introduction and significantly reduce the impact of invasive alien species on land and water ecosystems and control or eradicate the priority species".

1.2 Managing insect pests

Forest insect pests can be managed by the deployment of resistant or tolerant germplasm and by chemical, cultural and biological means. These various methods can be applied compatibly as part of integrated pest management (IPM) – a widely accepted approach implemented in both natural and planted forests worldwide (see section 3.4).

1.3 What is biological control?

Biological control involves the use of "natural enemies" as an essential component of IPM. In a broad sense, a natural enemy is an organism that causes the death or impairment of another organism. Biological control can be defined as using living natural enemies ("beneficial organisms") to control other living organisms ("pests"). International Standard for Phytosanitary Measures (ISPM) 3 defines a biological control agent (BCA) as a "natural enemy, antagonist or competitor, or other organism, used for pest control". The objective of all biological control programmes in forestry and agriculture is to reduce

the impacts of pests to below acceptable thresholds. Biological control can be used alone or in combination with other control methods in IPM programmes.

There are three main approaches to biological control:

1. *Augmentation biological control* – increasing the density of native or non-native natural enemies with regular releases. Releases may be made
   - at the beginning of each season, when relatively few individuals of a natural enemy are released (these are expected to reproduce during a certain period), or
   - as a single mass release of a natural enemy expected to result in immediate control.
2. *Conservation biological control* – the manipulation of habitat with the aim of enhancing the reproduction, survival and efficacy of natural enemies already present in an affected area.
3. *Classical biological control (CBC)* – the introduction of a natural enemy of non-native origin to control a pest, usually also non-native, with the aim of establishing a population of the natural enemy sufficient to achieve the sustainable control of the target pest.

1.4 Classical biological control

CBC is often a key component of IPM in forestry. It consists of controlling non-native pests by importing natural enemies (parasitoids, predators or pathogens) from their countries of origin. The objective is to establish permanent, self-sustaining populations of natural enemies that will disperse and suppress a pest population or reduce its rate of spread, including as part of wider IPM systems. Although, on its own, a CBC programme will not eradicate an invasive pest species, it can help reduce the population to below an acceptable threshold of damage. CBC is particularly well-suited as a management tool for non-native pests that invade new environments and can provide long-term, efficacious control at minimal ongoing cost.

1.5 About this guide

This guide has been written to enable forest-related personnel in economically developing countries to implement successful CBC programmes. It provides general theory and practical guidelines, explains the "why" (background and understanding) and "how" of forest pest control using CBC, and features 11 case studies of the successful application of CBC worldwide. CBC can be used to target many kinds of organism, including invasive plants and pathogens. This guide is concerned with forest insect pests, although its recommendations may also be valid for agricultural ecosystems.

Chapter 2 provides an introduction to some of the basic elements of a CBC programme. Chapters 3–5 examine the three main stages of a CBC programme, focusing especially on aspects of planning, implementation and the monitoring and evaluation of outcomes. Chapter 6 canvasses other important considerations, and Chapter 7 presents case studies. A glossary provides definitions of many of the technical terms used in this guide, and an annex lists the main species and genera of pests, BCAs and tree hosts described herein.

This guide is designed to complement a key FAO Forestry Paper, *Guide to the Implementation of Phytosanitary Standards in Forestry* (FAO, 2011), and the International

Standards for Phytosanitary Measures, especially ISPM 3: *Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and Other Beneficial Organisms*. Both these documents, which are available free of charge, should be consulted in tandem with this guide.

A female of the parasitoid Torymus sinensis *on a gall*

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2 Classical biological control

2.1 The benefits of classical biological control

CBC is a permanent, self-sustaining form of pest control. These qualities give CBC an important advantage over chemical and silvicultural pest-control methods as well as over the augmentation and conservation biological control methods. Once established, non-native natural enemies of targeted pests should require no further intervention, thus potentially resulting in substantial cost savings and economic benefits. For example, the CBC project against the mango mealybug in Africa generated an estimated cost–benefit ratio of 1:808 over 40 years (case study 7.7).[1]

Successful CBC projects enable reductions in the use of insecticides, which has substantial benefits for human health and the environment. CBC programmes are increasingly being developed to protect biodiversity and natural ecosystems where the use of pesticides is not an option – such as in the case of the orthezia bug on the island of Saint Helena (case study 7.4). CBC is also being used in protected areas – such as the Galapagos Islands, where the vedalia beetle (*Rodolia cardinalis*) has been introduced to help manage the impact of cottony cushion scale (*Icerya purchasi*), a plant pest.

2.2 Natural enemies used in classical biological control

In CBC, one or more of a pest's natural enemies are selected as potential BCAs. They may be parasitoids, predators or pathogens (Box 1), and they are most often sourced from a pest's native range, although related taxa from other invaded regions or elsewhere may also be considered.

BOX 1

Categories of natural enemies used in classical biological control

Parasitoids are parasitic organisms (mostly insects in the orders Diptera and Hymenoptera) that develop in a single host, usually killing it. Parasitoids can parasitize all insect developmental stages, from eggs to adults. They make up most of the agents used in classical biological control (CBC) programmes because they tend to be more host-specific than predators and

Box continues on next page

[1] Note that it is usually easier to calculate the economic benefits of CBC for agricultural pests than for forestry pests, partly because measuring differences in tree growth rates or timber production over relatively long rotations requires considerably more investment and time compared with measuring the growth of annual agricultural crops or the loss of fruit production.

Box continued

pathogens. For example, the winter moth in Canada has been controlled successfully through the introduction of a hymenopteran parasitoid, *Agrypon flaveolatum*, and a dipteran parasitoid, *Cyzenis albicans* (case study 7.9 describes this CBC programme; see case studies 7.3, 7.5–7.7 and 7.9–7.11 for other examples of the use of parasitoids in CBC).

Predators are entomophagous insects that feed on more than one prey in their lifetime. Larvae, nymphs and adults of many insect orders can be predators and may feed on all developmental stages of their prey. Currently, predators tend to be less-favoured than parasitoids in CBC programmes because their wider host ranges increase the risk of non-target effects. Nevertheless, there are many exceptions, and several specific predators have generated some of the biggest successes in CBC against tree pests, such as *Rhizophagus grandis* against the great spruce bark beetle (case study 7.2), *Hyperaspis pantherina* against the orthezia bug (case study 7.4) and the vedalia beetle against the cottony cushion scale.

Entomopathogens (viruses, fungi and microsporidia, and nematodes) have been used in CBC programmes to a much lesser extent than parasitoids and predators. An example is the nudivirus used against the coconut rhinoceros beetle (case study 7.1). Various groups of nematodes are well-known natural enemies of insects, but they are rarely used in CBC programmes. A notable exception is the deployment of a parasitic nematode against the sirex woodwasp (case study 7.8).

2.3 Communication and classical biological control

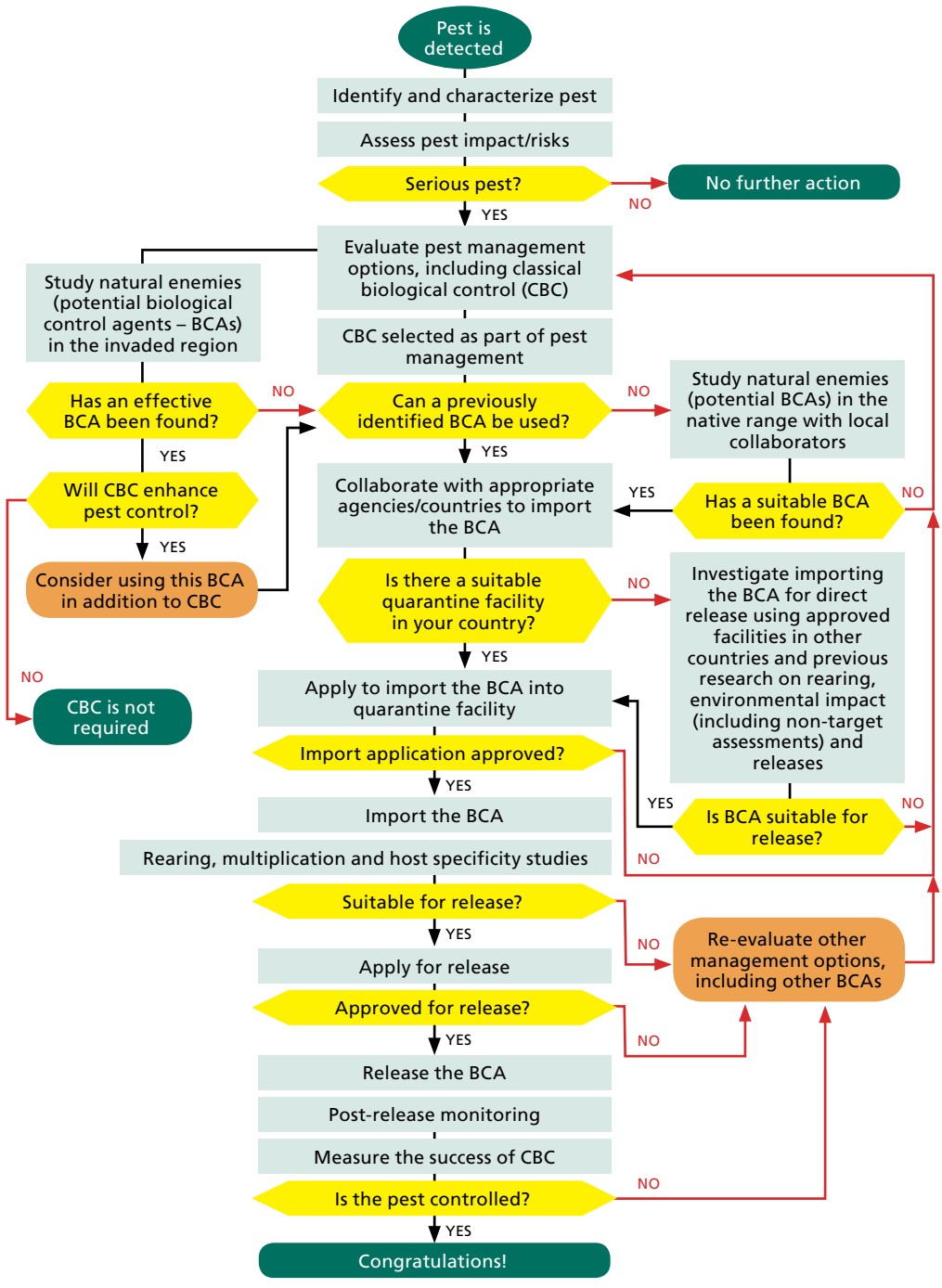
All CBC programmes should include awareness campaigns aimed at the programme's main beneficiaries (e.g. farmers, foresters and consumers). The objectives of such campaigns should be clear – such as to inform stakeholders about the intention to implement CBC and to provide updates on the process. It is important to initiate awareness campaigns very early on – as soon as the decision has been made to use CBC. Awareness campaigns must be maintained throughout the duration of a CBC programme using appropriate media to reach all affected stakeholders, including the public.

2.4 The classical biological control process

CBC programmes have two main phases (Figure 1):

1. the process to develop a CBC programme (described in Chapter 3); and
2. the implementation of a CBC programme (described in Chapter 4; see also Chapter 5 on monitoring and evaluation, which are parts of implementation).

FIGURE 1
Stages, actions and decisions in a classical biological control programme



Pinus radiata *plantation, New Zealand*

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3 Process leading to classical biological control

3.1 Pest detection

Globally, a range of phytosanitary measures is employed to limit the risk of accidental introductions of new invasive alien pests. One approach to managing high-risk pathways for such introductions is to implement ISPM 15: *Regulation of Wood Packaging Material in International Trade*, which is a phytosanitary standard designed to minimize the movement of pests in wood packaging. The FAO publication, *Guide to the Implementation of Phytosanitary Standards in Forestry* (FAO, 2011), outlines the most applicable phytosanitary practices in forestry.

Despite such measures, however, the potential remains for the introduction of invasive alien pests into countries and regions where they have not occurred previously. Such introductions, when detected, are the usual targets of CBC programmes.

Surveillance

Pests may be detected using active or passive surveillance. Active surveillance comprises structured, systematic and targeted surveys of trees and forests by trained personnel. Passive surveillance relies on reports of unusual signs, symptoms or pest insects on trees or in forests by stakeholders such as farmers and forest workers. Surveillance should target three main locations:

1. borders;
2. post-border areas; and
3. plantations, natural forests and urban forests.

Borders. Border surveillance involves inspecting goods arriving in a country for signs of pests (or the symptoms of these). Due to the large volumes involved, only a small proportion of such goods can be inspected. Even so, interception records are valuable indicators of which pests are moving around the world, the frequency at which they are moving and the pathways they are using. This knowledge helps improve pre-border phytosanitary measures, import protocols and post-border surveillance.

Post-border areas. Targeted surveillance can be carried out at high-risk sites (e.g. seaports, airports, import warehouses, container and pallet depots, and botanic gardens) using, for example:

- insect traps (e.g. those using pheromones or other lures, sticky traps, and light traps);
- sentinel plants (i.e. plants cultivated and located to attract known or new pests or diseases); and

- intensive ("blitz") surveys of insects and pathogens on trees and other woody plants along transects at high-risk entry sites.

Plantations, natural forests and urban forests. In many forests, surveillance is carried out as a routine part of management to assess forest health and to detect outbreaks of endemic or new pests. Methods include:

- walk-through surveys (along transects);
- drive-through surveys;
- aerial surveys (fixed-wing aircraft, helicopters and, increasingly, unmanned aerial vehicles – drones); and
- remote sensing (from aircraft or satellites).

ISPM 6: *Surveillance* describes general surveillance and specific surveys and stipulates the necessary components of surveys and monitoring systems for the purposes of plant-pest detection.

Actions following detection

Reporting. If a suspected new exotic pest is detected, an essential first action is to report this to the relevant national or provincial plant protection or biosecurity agency. ISPM 17: *Pest Reporting* describes how this should be done. Such reporting will trigger official responses and notifications.

Response plans. A regulatory framework that includes preparedness and response plans for pest incursions is highly desirable. Response plans can be generic or pest-specific: Australia's "PLANTPLAN" response plan developed by government and industry (Plant Health Australia, 2017) is an example of good practice for a generic approach.

Early responses under a response plan typically include:

- delimiting the surveillance area to determine the distribution of the pest;
- convening an expert panel to make early management decisions based on knowledge of the biology, ecology and economic importance of the pest, public awareness and contingency plans; and
- restricting the movement of potentially pest-infested goods to prevent the pest's spread.

The next step is to decide whether eradication is feasible or if another option, such as containment to limit the spread of the pest, is more appropriate. The earlier a pest is detected, the higher the probability of eradicating it, containing it or limiting its spread.

3.2 Pest identification and characterization

Identification

As soon as a potential insect pest is detected, it is important to identify it correctly to determine whether it is:

- new to the country or region;
- a native species; or
- an already-established non-native species.

This means it is essential to know which insects are present locally. Such information might be obtained from local historical records of pest occurrence; scientific literature; previous surveys of forest pests; and the local knowledge of forest growers and tree owners.

Images and specimens of potential new pests can be directed to local taxonomic experts, if available, for rapid identification, and they can also be sent to international experts. Resources required for local identification include:

- access to good-quality microscopes (ideally fitted with cameras for taking high-quality images); and
- taxonomic keys relevant to the insect involved.

Many online resources are available to assist with provisional pest identification, including Internet image searches (e.g. Forestry Images, PaDIL and online insect identification keys);[2] email discussion groups such as PestNet;[3] and software applications ("apps") for mobile devices.

If experienced morphological taxonomists are unavailable to make authoritative diagnoses, molecular barcoding is the next best option for identification. This requires access to a molecular-biology laboratory and experienced staff to carry out analyses and interpret results. If such facilities and personnel are unavailable in-country, appropriately preserved specimens can be sent internationally for analysis as per national protocols. An advantage of molecular-based diagnoses is that they may also be able to identify a pest's region of origin, which could be important for a biological control programme.

Characterization

Characterizing a new pest means documenting its biology, ecology and known management options. Early characterization can be obtained by reviewing the scientific literature, which could contain detailed relevant information if the insect is a known pest. If the insect is not well known, scientific literature might exist for closely related species that share similar biological traits.

Crucial information needed for assessing risk and management options includes:

- Host range
- Life-cycle duration
- Global distribution
- Climatic requirements
- Dispersal ability
- Control methods
- Nature of damage or symptoms on host
- Associated natural enemies.

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[2] "Forestry Images" is an image database for forestry species of economic concern maintained by the Centre for Invasive Species and Ecosystem Health at the University of Georgia, United States of America; it is available at www.forestryimages.org. "PaDIL" – the Pest and Diseases Image Library – is an image database and information tool for biosecurity and biodiversity maintained by the Australian Government's Department of Agriculture and Water Resources; it is available at www.padil.gov.au.

[3] "PestNet" is an email network to provide rapid advice and information on crop protection, including the identification and management of plant pests; it is available at www.pestnet.org.

3.3 Pest risk analysis

When a new insect is detected, a pest risk analysis (PRA) is conducted to:

- determine whether the organism is a pest and is likely to establish and spread in the country;
- evaluate whether and to what extent the pest has the potential to cause economic, social and environmental damage;
- evaluate the potential impacts on international trade; and
- determine any phytosanitary measures to be taken against it.

A PRA will provide sufficient information to determine whether the newly detected insect is a significant pest that should be managed. It is important, therefore, to determine the risk posed by an insect as soon as possible after it is detected. ISPM 2: *Framework for Pest Risk Analysis*, ISPM 11: *Pest Risk Analysis for Quarantine Pests*, and ISPM 21: *Pest Risk Analysis for Regulated Non Quarantine Pests* describe the processes for PRA and for selecting appropriate risk management options, and they provide information on assessing plant-pest risks to the environment and biodiversity. Some regional plant-protection organizations (e.g. the European and Mediterranean Plant Protection Organization) and countries (e.g. Australia and New Zealand) have specific PRA processes. A country's national plant protection organization (NPPO) or delegated authority should conduct PRAs for newly found non-native insects.

A PRA requires an understanding of the ecology and behaviour of the insect, including the range of suitable hosts and its life stages, method and rate of reproduction, life-cycle duration and climatic requirements. PRAs consider the potential impacts of pest damage on forest plantations, including losses in nursery production, total crop losses, reduced growth rates, poor tree form and decreased wood quality. In native forest ecosystems, negative impacts on tree species may cause changes in forest composition and a consequent loss of habitat for wildlife dependent on affected tree species.

Where possible and practicable, estimates should be made of production losses. These are relatively easy to make when damage is severe (e.g. the total loss of a plantation) and more difficult if damage has subtler effects on growth rates and wood quality. Where the impact is on growth rates, impact assessments may be needed over several years because growth losses may take some time to be realized and many tree species have significant capacity to compensate for defoliation.

The scientific literature and historical records of pests detected in other countries or regions may provide much of the information needed for conducting a PRA. It is especially important to obtain information from other invaded areas because the actions of natural enemies may suppress an insect pest in its native range.

Key considerations that can help initiate a PRA include the following:

- Is the insect invasive in other countries?
- Is the insect a significant pest in other countries or regions or in its native range?
- Is the insect included in national and international pest lists?
- Have previous PRAs been conducted in other countries?
- Which tree host(s) has the insect affected?
- How severe is the damage and does this vary according to host species?

- What is the insect's current distribution?
- Is the distribution or range increasing?
- Are response plans available in other countries?
- What methods are used to control the insect in other countries?

3.4 Evaluating pest management options

If a PRA concludes that the risk is high enough to take action, options such as eradication, containment and management should be evaluated to determine which is most appropriate. Note that a combination of several management methods can be used in an IPM approach.

Eradication

The eradication of a new pest may be feasible if surveys show it has a limited distribution. Other aspects to consider are the pest's dispersal and reproductive ability and the availability of control methods with a high probability of eliminating the pest. Such methods may include:

- effective chemical and biopesticide treatments;
- traps that can be baited with pheromones or kairomones (see "semiochemicals" below) that efficiently trap the pest and reduce numbers quickly; and
- especially for bark- and wood-boring pests, the removal and destruction of infested trees.

More information on pest-eradication strategies is available in ISPM 9: *Guidelines for Pest Eradication Programmes*.

Containment

If a pest is not eradicable or eradication fails, containing the pest may be the next-best option. The objective of containment is to prevent the pest from expanding its range or at least to slow its spread to other areas. Methods usually focus on limiting human-assisted spread, for example by restricting the movement of nursery material and timber products. Methods for eradicating a pest can also be used for its containment.

If a pest cannot be eradicated or contained, other management methods should be evaluated.

Integrated pest management

IPM is the combination of prevention, monitoring and suppression methods to sustainably maintain pest populations at an economically, socially and environmentally acceptable level. IPM may involve carefully selected and applied chemical, silvicultural and biological control practices.

For IPM to be effective, field practitioners need a reasonable knowledge of the biology and ecology of the pests and host trees involved. They also need a good understanding of the control methods used in forestry, as described below.

Chemical control

Insecticides are commonly used worldwide as a control method in forestry. Increasingly, however, chemical use is being restricted due to its negative impacts on the environment and human health. Forest sustainability certification schemes, such as that of the Forest Stewardship Council, impose strict conditions and restrictions on chemical use by forest managers seeking certification for their products. "Soft" insecticides (those with few off-target impacts) can still form an important part of IPM systems when used appropriately. Evaluations should determine:

- which chemicals may be effective against the new pest;
- whether those chemicals are registered for use in forestry; and
- whether the use of such chemicals is permitted under the Forest Stewardship Council and other certification schemes.

Emergency-use registrations can sometimes be obtained for eradication programmes. The Joint FAO/World Health Organization (WHO) Meeting on Pesticide Residues and the Joint FAO/WHO Meeting on Pesticide Specifications have generated a list of evaluated pesticides.[4]

Silvicultural control

Some pests can be managed effectively by altering silvicultural practices; this requires a good understanding of interactions between a pest's biology and silvicultural practices. Changes in silvicultural practices may include removing trees that are current or imminent sources of infestation and altering the timing of planting, thinning, pruning or harvesting. Interactions between soil nutrition and the application of fertilizers may also be important. Decisions on silvicultural pest-control practices require close collaboration between entomologists and silviculturalists, who should jointly determine which manipulations are likely to be effective and whether implementation is practical.

Tree resistance or tolerance

Any resistance to or tolerance of a new pest among tree hosts should be identified as early as possible. Selecting for genetic resistance in tree populations, and subsequently growing resistant varieties, can be an effective method for managing new pests. On the other hand, the developers of resistant germplasm often retain ownership rights to this genetic material, which therefore may not be readily available to small-scale growers, at least in the short term.

The time it takes to identify resistance to or tolerance of a new pest, develop the germplasm and deploy the plants will depend, to some extent, on the age at which trees become susceptible. For example, resistance to a pest that attacks seedlings can be screened for and deployed much faster than resistance to a borer pest that attacks older trees. Genetic markers of resistance or tolerance can be used to speed this process, but this will require significant investment in research.

[4] The list is available at www.fao.org/agriculture/crops/thematic-sitemap/theme/pests/jmps/ps-new

Semiochemicals

The methods insects use to find mates and locate hosts can be exploited in management programmes. Pheromones (insect-produced chemicals that affect the behaviour of individuals of the same species) and kairomones (chemicals produced by one species that influence the behaviours of other species) are used widely to manage pests. Pheromones, which are species-specific, are an especially useful tool for monitoring insect populations and reducing population numbers. Suitable chemicals may already be available for certain well-known pests but, for new pests, discovering, testing and commercializing behavioural semiochemicals can be a long and expensive process.

Literature searches and expert advice will ascertain whether potentially effective semiochemicals are available for monitoring and regulating populations of a new pest (e.g. Pherobase is a good resource for semiochemical information).[5] Effective semiochemicals (as well as suitable traps and methods for applying the chemicals) are available for some common invasive forest pests, with several companies specializing in such products.

Biological control

Biological control – the use of natural enemies to regulate pest populations – is a frequently employed and often-effective management practice. There are three main approaches, which mostly apply either to already-existing endemic or exotic pests or to newly introduced non-native pests:

1. Conservation biological control
2. Augmentation biological control
3. Classical biological control.

Conservation biological control. Cultural practices that conserve existing thriving natural enemies in an ecosystem by providing them with suitable conditions include, for example:

- encouraging nectar sources for parasitoids to enhance their survival and reproductive capacity;
- providing habitats in which natural enemies can survive during unfavourable conditions (e.g. after a crop has been harvested), such as by promoting the growth of plant hosts that help maintain natural-enemy populations; and
- reducing the effect that pesticides have on natural enemies, for example by switching to biopesticides or reducing pesticide dosages.

Augmentation biological control. This approach involves continually rearing, multiplying and releasing natural enemies of a pest – generally in large numbers at the same location repeatedly. Augmentation biological control is usually used when natural control systems are ineffective and other methods (e.g. the use of chemicals) are undesirable. Natural enemies used in augmentation biological control are often supplied commercially by specialized businesses. This approach also includes the use of entomopathogens as biopesticides.

Classical biological control. CBC involves importing natural enemies from a pest's endemic range and releasing them to become established in the new country or region as a means of providing ongoing pest control. This method is the focus of this guide.

[5] Pherobase is available at www.pherobase.com

3.5 Studying natural enemies in an invaded region

In evaluating the options for IPM, early surveys of a pest will have determined whether natural enemies are already present and having a significant impact on the pest population. Such natural enemies may be from the endemic range of the pest that have travelled with it, or they may be organisms endemic to the invaded region that are exploiting the new pest as a host or prey (Box 2).

BOX 2

Studying natural enemies in an invaded region

In a classical biological control (CBC) project, it is important to study the natural-enemy complex already present in the area of introduction to identify any native natural enemies that could control the invasive insect pest. Such natural enemies may be native species that adapt to progressively attack the insect pest, or they may be species that have been introduced unintentionally from the pest's region of origin. In both cases, natural enemies could reduce insect pest populations substantially or at least achieve satisfactory control (see, for example, case study 7.11). Observations of native natural enemies in the area of introduction should be carried out frequently because it may take several years or more for native enemies to adapt to invasive insect pests.

Knowing which natural enemies attack a pest in an area of introduction helps in selecting the non-native natural enemies to introduce through a CBC programme. For example, if a native parasitoid of a pest's eggs is already abundant in the introduction range, the focus could be on introducing a non-native parasitoid or predator of the pest's larval stages. This would avoid introducing a natural enemy that would occupy a similar niche to an abundant natural enemy.

There are three key tasks in studying natural enemies:

1. Complete a literature review of natural enemies on congeneric species or closely related species (insect pests and natural enemies).
2. Conduct a survey of natural enemies in the area of introduction and assess their roles in the mortality of the insect pest.
3. If natural enemies are detected with potential to control a pest but little is known of their biology, ecology or efficacy, conduct further studies of these organisms. Scientifically sound information from such studies plays an important role in CBC decision-making processes.

Knowing whether an existing natural enemy is effective will have an impact on decisions to import BCAs as part of a CBC programme. The presence of local natural enemies of a new pest also opens up opportunities for conservation and augmentation biological control.

A worker in a biological-control quarantine facility

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4 Implementing classical biological control

When CBC is selected as the biological control method, the next step is to determine a suitable BCA, which must meet import and quarantine regulations before it can be reared and released.

4.1 Reviewing potential biological control agents

Any CBC programme should be based on a thorough understanding of similar previous programmes against the target pest and of the natural enemies that could be used as BCAs.

Review previous and existing biological control programmes

A comprehensive literature review should be carried out on previous and existing biological control programmes for the target pest and any known natural enemies of the pest in other parts of the world. Given that pests often invade more than one area, several CBC programmes for a target pest may already exist (Box 3). Much can be learned from these, but bear in mind that the same pest may need different control measures in different areas. Nevertheless, obtaining as much information as possible from previous and existing CBC programmes and potential BCAs will undoubtedly help in planning the new CBC programme and in minimizing costs.

BOX 3

Classical biological control projects against the same insect pests in different countries

There has been a marked increase in recent years in the number of eucalypt-pest introductions and in the speed at which such pests are moving between countries and continents. This is posing significant challenges for classical biological control (CBC) practitioners in affected countries, stretching their resources to manage multiple CBC programmes simultaneously.

Recent examples include:

- the gall wasp (*Leptocybe invasa*), which was first found in the Middle East in 2000 and had dispersed to every continent that grows eucalypts within ten years; and
- the bronze bug (*Thaumastocoris peregrinus*), which was first reported in South Africa in 2003 and by 2016 had spread consecutively to South America, Europe, New Zealand, the Middle East and North America.

Box continues on next page

Box continued

The Biological Control of Eucalypt Pests Alliance is helping coordinate and execute surveys of natural enemies, identify candidate biological control agents and make them available to CBC programmes in partner countries. The Alliance's research has focused on five eucalypt pests of Australian origin: eucalyptus weevils (*Gonipterus* spp.); the red gum lerp psyllid (*Glycaspis brimblecombei*); two eucalypt gall wasps (*Leptocybe* spp. – see case study 7.5 – and *Ophelimus* spp.); and the bronze bug (*Thaumastocoris peregrinus* – see case study 7.6).

The literature review should cover all the information available on CBC against the target pest, including primary publications and reports, and it should consult CBC project databases worldwide. Every piece of information that may be relevant to the planned CBC programme should be examined, including natural-enemy complexes in various regions, natural-enemy selection processes and rearing and release methods, and programme outcomes. The strengths, weaknesses and challenges of previous programmes should be assessed.

If no information exists on previous CBC projects carried out against a target pest, published information on the target pest's natural enemies should be reviewed. This will assist surveys for natural enemies in the native range, and investigations may also provide opportunities for collaborating with experts in CBC against the pest and obtaining access to supplies of BCAs. Collaboration might also be possible with experts in the native range, who could provide support for surveys and expertise in other relevant areas.

Preliminary investigations may lead to one of the following three main findings:

1. *Previously successful CBC programmes exist* – when a previous CBC programme has been successful, the option of introducing the same natural enemy through the planned programme may be considered if:
   - the environmental conditions in the previous programme were similar to the conditions of the planned CBC programme (e.g. similar climate and habitats); and
   - analysis of the previous programme suggests that the most suitable natural enemy was chosen.

   In this case, surveys and studies in the native range (see section 4.2) may be unnecessary. Nevertheless, the potential for non-target effects should be considered because biodiversity in the planned area of introduction may differ from that in the previous CBC programme.
2. *Previous studies concluded that no suitable natural enemies exist* – if comprehensive surveys and studies to find a BCA conclude that no suitable natural enemy exists in the native range, discontinuing the planned CBC programme should be considered.
3. *More information is needed about natural enemies as potential BCAs* – in all other cases (for example, CBC has not previously been carried out against the pest, previous CBC programmes were poorly executed, or the environment in the new target area differs significantly from those previously targeted) the planned CBC programme should continue with surveys of natural enemies in the native range.

4.2 Studying natural enemies in a pest's native range

Purpose

Understanding the natural-enemy complex of a target pest in its native range (and especially the respective roles of natural enemies in controlling pest populations) will help in selecting the most efficient and safe BCAs to introduce to the infested area. This knowledge may already be available for some target pests, for example from previous CBC programmes in other parts of the world. If the natural-enemy complex is not well known, however, a study is a key requirement. It may also be the most time-consuming and expensive step in the entire CBC programme.

Collaborate closely with local agencies

Studying the natural-enemy complex in its native range must always be done in close collaboration with relevant national institutions, such as universities, research institutes and government agencies. These institutions can provide logistical support and scientific collaboration, and they can share the knowledge generated, for example through joint publications and the sharing of voucher specimens. All studies in the native range should adhere to the laws of the surveyed countries, especially in relation to the sampling and exporting of insects and plant materials. The NPPO of the country of survey should be the first point of contact for information on exporting insects, other natural enemies and plant materials.

Survey a relevant sample of the pest's native range

Because a pest's native range is often large and not clearly defined, it is usually not possible to survey its entirety. The main survey areas for a natural-enemy complex should be selected using the following two criteria:

1. *Survey areas similar to the area of introduction* – surveys should concentrate on areas that are climatically and ecologically most similar to the intended range of introduction (or the target area, if the CBC programme will focus on only a part of the introduction range). Computer programmes such as CLIMEX can be used to help determine similarities in climatic conditions ("matching") and the likely effects on species in the area where the natural enemy is to be introduced.
2. *Survey the pest's area of origin* – where possible, surveys should focus on the putative (presumed) point of origin of a pest. The true "native range" is difficult to define, however, because the pest may have invaded areas many years previously, and this "naturalized range" may be difficult to distinguish from its native range; this is particularly the case where host trees have been planted outside their native ranges. For example, the boxwood tree moth (*Cydalima perspectalis*), an invasive species in Europe and the Caucasus, is thought to be native to China, where it occurs on ornamental boxwood trees (*Buxus* spp.) in most provinces. Because natural stands of boxwood trees occur in only a limited area, however, other Chinese provinces are considered to be invaded areas and only part of the original natural-enemy complex may be present there. In this case, surveys of natural

enemies should be conducted in natural stands of boxwood or in ornamental boxwood trees in the same areas.

In some cases, a pest's area of origin can be determined through genetic studies using molecular tools. If the area of origin remains unknown, however, surveys of natural enemies should focus on the most likely areas of origin, especially those that are climatically and ecologically similar to the target area of the CBC programme.

Objectives of natural-enemy studies in pest's native range

Survey natural enemies in the field and quantify their impacts on the pest. All life stages of a pest should be collected in the field and reared in the laboratory so that naturally occurring parasitoids and pathogens might be found. Methods will differ, depending on the target insect pest and its developmental stages; researchers should be encouraged, therefore, to consult the scientific literature related to the relevant insect group and its natural enemies.

Parasitoids are often the easiest natural enemies to obtain and quantify (compared with predators and pathogens) because they can be reared from material collected in the field. Apparent rates of parasitism can be measured by dividing the number of emerged parasitoids by the sum of the number of hosts and parasitoids. Note that there is potential for error in this method. The results will be influenced by the timing of collection of the parasitized hosts, which is related to:

- the time during which the hosts were available to be parasitized; and
- the proportion of hosts and parasitoids that would have already emerged.

This error can be reduced by repeated sampling at different times.

Pathogens of insect pests can also be identified through field collections. Quantifying the mortality caused by such pathogens is unreliable if based solely on field collections, however, because excessive mortality may occur in laboratory rearing. Some pathogens do not kill their host insects directly but reduce measures of fitness such as longevity and fecundity. Such factors can only be assessed in specific laboratory studies, which are rarely part of CBC programmes (on the other hand, programmes for the sirex woodwasp routinely assess the influence of the entomopathogenic nematode BCA, *Deladenus siricidicola*, on the woodwasp's fertility – see case study 7.8).

Predators are the most difficult natural enemies to study because they rarely leave signs of attack when they eat or kill their prey. Identifying predators requires careful observation, sampling in the field, and further testing in the laboratory. Quantifying the effects of predators on target pest populations is even more challenging.

What level of detail is required to study the effects of natural enemies? Quantifying the exact effects of natural enemies on pest mortality can be done using the life-table method. In this approach, all developmental stages are studied separately in field conditions and the various mortality factors are quantified for each stage. Life tables are useful for comparing the effects of mortality factors in the native and introduction ranges, but their use is very time-consuming and their efficiency varies by insect group. For these reasons, life-table studies are rarely carried out in CBC programmes. Note that it is usually unnecessary to gather accurate data on the mortality caused by natural enemies

in the native range because mortality rates tend to vary substantially between the native and introduction ranges. Thus, identifying apparently important natural enemies in the native range is usually sufficient for their further evaluation.

Study the biology and ecology of pests and natural enemies in their native ranges. The successful introduction of a BCA requires in-depth knowledge of the biology and ecology of the pest and its natural enemies. This knowledge is gained through a combination of literature reviews and field and laboratory research. Biological and ecological studies of pests and natural enemies will be more relevant and valid if done in the field or in non-quarantine laboratory conditions in the native range rather than in the constrained conditions of a quarantine laboratory in the introduced range. Knowing the biological and ecological characteristics of natural enemies helps in assessing whether a parasitoid, predator or pathogen will adapt to the pest and environment in the introduction area. The availability of this information will also greatly simplify studies conducted in quarantine conditions in the introduction area or elsewhere.

Selecting which studies to conduct will depend on what is known about the pest and its natural enemies and how easy it is to do field work in the native range. Potential aspects for study include the following:

- *Life cycle of pest's natural enemies and other potential hosts or prey* – this type of study may help determine the specific stage(s) at which a natural enemy attacks or kills a pest host or prey. It may also identify the natural enemy's fecundity, pest resistance, pre-oviposition period, development time, host-stage suitability and preference, and intraspecific competition, as well as any potential non-target effects.
- *Climatic requirements of natural enemies* – climatic requirements are assessed through field surveys in climatically different areas and also in laboratory studies of a natural enemy's development under differing temperatures and humidities and its cold and heat hardiness. In some CBC programmes, studies of climatic requirements generate climate models for predicting establishment success for a natural enemy in the introduction area. Climatic requirements can also be modelled or predicted from location data in previous collections, including data from museum specimens.
- *Habitat preferences of natural enemies* – field surveys can determine a natural enemy's habitat preferences at the scale of both macrohabitat (e.g. humid versus dry zones) and microhabitat (e.g. preferred host plant species for parasitism).
- *Associated cryptic species and biotypes* – it is now clear that many pests and their natural enemies once thought to belong to single species actually comprise several cryptic species or biotypes of the same species. Such species or biotypes may differ in important traits such as host range, host preference, climatic requirements and fecundity. Thus, it is essential to select the most appropriate species and biotypes for further testing and potential introduction. Characterizing cryptic species and biotypes can begin in the native (indigenous) range using three interdependent selection approaches based on collections of several populations from different regions, hosts or habitats:

1. Test the different populations of species or biotypes for traits such as climatic requirements and host specificity (or preference) in the laboratory.
2. Sequence the different populations using molecular approaches (barcoding) to detect different lineages.
3. If cryptic species are suspected, perform crossings to assess their reproductive compatibility.

- *Elements of population dynamics* – a natural enemy's potential to control a pest at various pest densities can be assessed by determining the host–parasitoid, host–pathogen or prey–predator density dependencies.

Studies on rearing natural enemies and host-specificity assessments are usually conducted in quarantine facilities in the introduction area (see sections 4.4 and 4.5). It is also possible, however, to gather important information on these aspects in the native ranges.

4.3 Importing biological control agents

This section describes the process for importing BCAs to manage forest insect pests, as set out in ISPM 3: *Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and Other Beneficial Organisms*. ISPM 3 lists the principles for facilitating safe trade and using BCAs and describes the responsibilities of an exporting country's NPPO, the exporter and importer, and the importing country's NPPO before, during and after importing a BCA.

Import and export requirements for biological control agents

Before importing or exporting a BCA, both the importer and exporter should contact their respective NPPOs or other relevant authorities to ensure they understand the specific requirements for moving the BCA safely from one country to another.

Import requirements. In most countries, the importing process cannot begin until an import permit or licence has been issued to the importer. A permit or licence is the official authorizing document that describes the import requirements, as developed by the importing country's NPPO based on the results of a risk assessment. If the risk of importing a BCA can be managed adequately, the importing country's NPPO will issue the permit or licence to allow the BCA to be brought in, provided that the consignment meets the specified requirements. Some countries may require evidence of a risk assessment and cost–benefit analysis before granting a permit to import a BCA into containment.

Export consignment requirements. A BCA consignment for export is prepared according to the import requirements of the import permit or licence and any other regulatory conditions. In some countries, exporters may need to obtain export permits issued by the relevant agency in the exporting country before exporting BCAs. The relevant agency in the exporting country will need to be satisfied that a consignment meets all requirements before issuing the relevant certification, such as a phytosanitary certificate or an export permit.

Transporting biological control agents

Transporting a BCA between countries can be very challenging given differing rules and regulations of the importing and exporting countries (and, potentially, of transit countries). It is crucial to check with all countries involved before attempting to transport a BCA (Box 4).

BOX 4

Key considerations when transporting a biological control agent into an importing country

Protocols and documentation. Shipping and handling protocols must be well defined, clearly written and appropriate for the biological control agent (BCA).

Emergencies. An emergency response plan should be prepared in the event of a BCA escape (while in transit or from a quarantine facility).

Hand delivery. Unaccompanied consignments of BCAs may encounter transport delays and extra customs and biosecurity restrictions. It is often advisable, therefore, to hand-carry a BCA to avoid delays.

Air-delivery restrictions. Transporting BCAs between countries can be problematic because some courier companies do not allow the shipment of live materials such as BCAs.

Customs and biosecurity. To avoid unnecessary delays, accurate documentation for a BCA consignment should always be available. When a BCA is imported for research purposes, it may be necessary to include a detailed description of the nature of the material proposed for importation and the type of research to be conducted.

Point of entry to quarantine. After clearance at the point of entry (e.g. airport, mail receipt or hand delivery), BCAs must be moved directly and as quickly as possible to a designated quarantine facility.

Trial shipments. It is good practice to send a small batch of a BCA in advance to test the transport process.

Requirements for quarantine facilities at import destination

Quarantine facilities are used to ensure that imported BCAs are reared and studied in containment before permission is granted for their release.

There is no internationally agreed standard for establishing and maintaining quarantine facilities for rearing and multiplying BCAs. The European and Mediterranean Plant Protection Organization, the North American Plant Protection Organization, and countries such as Australia, Canada, Japan, New Zealand, the Republic of Korea and the United Kingdom of Great Britain and Northern Ireland have their own standards. Because legal requirements for the establishment, maintenance and use of quarantine facilities can differ between countries, it is essential to work closely with relevant NPPOs or other delegated authorities. In all cases, the relevant NPPO or other delegated authority is accountable for the initial approval and ongoing audits of quarantine facilities.

Quarantine facilities must have the necessary approvals in place before receiving BCAs. Box 5 sets out basic good-practice requirements and considerations for quarantine facilities containing insect BCAs.

BOX 5

Basic requirements for quarantine facilities for importing insect-pest biological control agents

- Secure facility
- Approval by the country's national plant protection organization and an audit inspection conducted
- Facility manager well-trained in the dos and don'ts of quarantine
- Double-door dark-room entrance
- Quarantine clothing (worn only inside the facility)
- Sealed facility to prevent escape (e.g. filters on ventilation systems, sealed spaces between windows and bricks)
- Operational procedures for removing waste and other items from the facility (e.g. autoclaves, incineration, freezers that can be set at -40/-80 °C)
- Negative air pressure in the facility so that air flows in, not out
- Recommended:
  - Air curtains at the entrance
  - Insect traps (e.g. light traps) in dark corridors and at the entrance to warn of any insects escaping from rooms

Quarantine facilities should be designed to accommodate containment rooms with special ventilation that can manage a wide temperature range, elevated relative humidity, and potential allergy problems related to insect-rearing. Some quarantine facilities also have separate greenhouses to provide adequate natural and artificial light for normal plant growth and insect behaviour.

Quarantine facilities are required to meet standard levels of physical containment (PC) depending on the nature of the BCA and the risk associated with it. Some countries specify the same PC levels as those used for biosecurity (BS). There are four generally accepted PC/BS levels reflecting the status of a quarantine facility and the systems in place to contain BCAs safely. PC/BS levels 1–3 apply mainly to quarantine facilities handling BCAs, and PC/BS level 4 is typically used for dangerous arthropods with implications for human health. Box 6 summarizes PC/BS levels 1–3 for quarantine facilities.

Establishing and maintaining quarantine facilities requires ongoing resources (the higher the PC/BS level, the more resources required). Where feasible, countries or regions should consider sharing facilities.

In the basic PC/BS level 2 quarantine facility depicted in Figure 2, entry and exit are via darkened airlocks 1 and 2 only. Note that all doors open inwards, creating a small

BOX 6

Physical containment/biosecurity levels for containing biological control agents securely

Different areas of a quarantine facility can be graded as providing a specific level of physical containment (PC) or biosecurity (BS). PC/BS levels 1–3 are considered adequate for the containment of biological control agents (BCAs); PC/BS level 4 offers additional biosecurity but is unnecessary for BCAs.

The PC/BS level may be increased by deploying more than one type of PC method; this is known as layering. For example, properly used insect cages can increase the containment level of an experiment within a facility, provided that screen dimensions and materials are appropriately matched to the BCA's size and level of mobility.

PC/BS level 1 (BSL-1) is suitable for work with BCAs (arthropods) already present in the local geographic region.

PC/BS level 2 (BSL-2) is the minimum containment level required for working with non-native BCAs (arthropods) that would be unviable or would establish only temporarily if they escaped. BSL-2 builds on the practices, procedures, containment equipment and facility requirements of BSL-1. It has more stringent requirements for physical containment, disposal and facility design. Access is also more restricted than for BSL-1.

PC/BS level 3 (BSL-3) is adequate for work with non-native BCAs (arthropods and pathogens) that would pose significant risks to the environment if they escaped. BSL-3 builds on the requirements of BSL-2: access is more restricted and pathological containment is more important in determining practices and facilities. It is recommended that areas for arthropod and pathogen research are separated within the quarantine facility.

Required standards for pathogen work can be met by improving aspects of existing containment provisions. This includes installing high-efficiency particulate air filters for exhaust air; directional airflow; air-pressure control; local pressure-differential gauges; alarms to the central building computer; fast, responsive, positive seal dampers; premium ceiling, wall and floor coatings; and the capability to decontaminate gas.

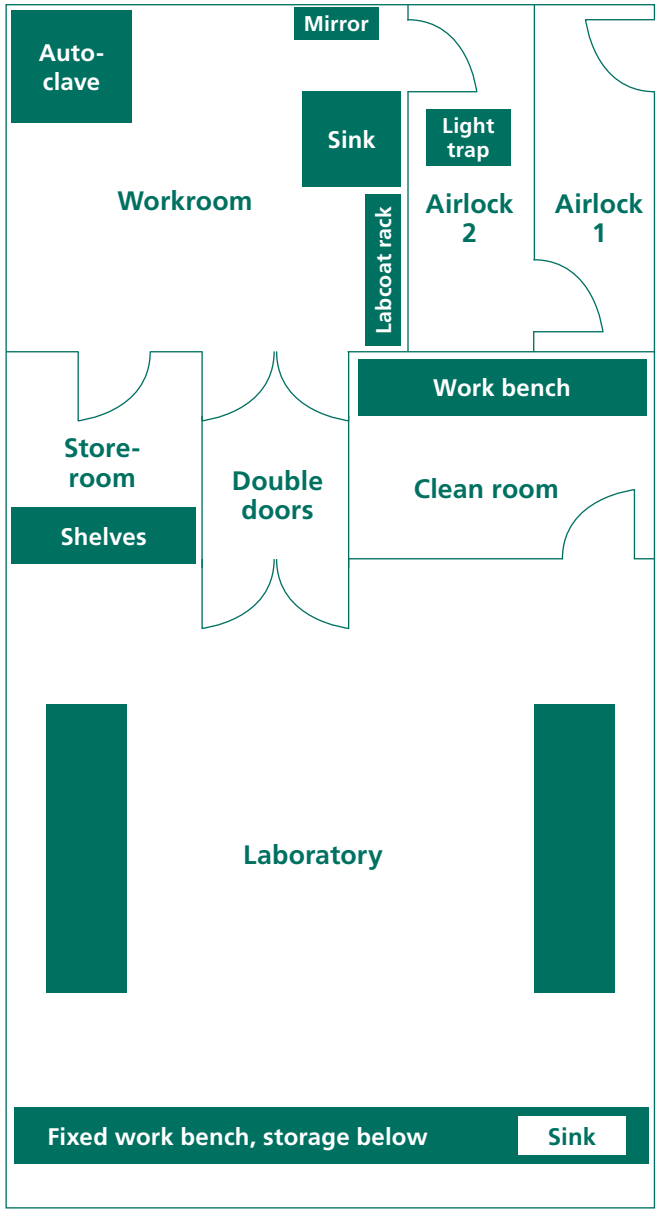
negative pressure when opened. The workroom contains an autoclave, which must be used to process all material before it is disposed of outside. The "clean room" is where consignments of BCAs are opened and processed before the BCAs are moved into the laboratory for breeding and testing.

Receiving biological control agents into quarantine

Consignments of imported BCAs, whether couriered or hand-delivered, must go directly to an approved quarantine facility before they are opened.

Once received into the quarantine facility, a BCA consignment can be opened and the BCAs removed. The survival and condition of BCA specimens should be recorded,

FIGURE 2
Floor plan for a basic physical containment/biosecurity level 2 quarantine facility



including the total number received, the percentage survival, the sex ratio, and comments on the apparent health of the specimens.

The contents of consignments should be assessed for contaminants – that is, other organisms that have been accidentally imported with a BCA, such as insects, hyperparasitoids, phoretic mites, nematodes and microbial pathogens of the BCA. This step is crucial: the introduction of contaminants into the release area could compromise the efficacy of the BCA (for example if the contaminants are hyperparasitoids or microbial pathogens of the BCA) or result in the accidental introduction of other pest species. Detected contaminants should be killed, identified and preserved as voucher specimens for future reference.

After all individuals of a BCA have been removed and the package examined for contaminants, all packing material should be destroyed according to quarantine operating procedures.

The identity of an imported BCA must be confirmed, where possible in consultation with appropriate taxonomic experts. It is good practice to use the BCA's molecular barcode sequence in combination with taxonomic expertise to confirm a consignment's identity, although this will only be possible if the species' barcode sequence is available. In some cases, taxonomic expertise may be unavailable, and barcode sequencing may be the only method of species confirmation. Box 7 lists key considerations when receiving BCAs into quarantine.

BOX 7

Key considerations for receiving biological control agents into quarantine facilities

- Designate a secure area for receiving, holding and opening consignment containers. Some countries require that such containers are handled in the presence of an officer from the national plant protection organization.
- Adhere to standard operating procedures for receiving, opening and handling consignment containers.
- Conduct a taxonomic validation study of the material using morphological or molecular methods.
- Keep voucher specimens of the imported biological control agent (BCA) for future reference.
- Complete quality assurance assays on the phytosanitary condition, health and viability of imported organisms.
- Always maintain good records and accompanying documentation that may be relevant to the export, shipment, import or release of a BCA.
- Consider making formal agreements between contracting parties to indicate, for example, that:

Box continues on next page

Box continued

  - the exporting party will not be responsible or liable for the failure of a BCA to become established or to control the targeted pest, or for any effect on non-targets; and
  - both parties can meet their respective import and export requirements.
- Detailed information is available in International Standard for Phytosanitary Measures No. 3.

The country's NPPO and other relevant authorities must be notified of receipt of a BCA consignment, along with confirmation of the species' identity and whether any contaminants were detected.

Receiving biological control agents for direct release

In some situations, an importing country may initiate a CBC programme without having its own in-country quarantine facility for rearing BCAs. In this case, it may be possible to import (and release) BCAs directly from participating laboratories or quarantine facilities in another country. To do so, it is crucial that the necessary permits, as well as research on rearing, environmental impacts (including non-target assessments) and releases conducted by the participating laboratories, are completed – in accordance with the requirements of the importing country – before the dispatch of a BCA to the importing country. Although this is not the ideal way to import a non-native BCA into a new environment, it can be done safely through carefully coordinated partnerships.

Transporting a live BCA requires packaging that prevents specimens of the BCA from escaping and maintains the consignment's viability. Where possible, a small test shipment of the BCA should be conducted to test the importation process. Arrangements should be made with relevant authorities to clear shipments promptly at the point of entry (e.g. an international airport). Consignments should be transferred to a suitable facility with minimal delay and placed in cages or screened greenhouses to be opened, sorted and studied.

Before a BCA is imported, the exporter should take the utmost care to ensure that consignments will not be contaminated (e.g. with hyperparasitoids, pathogens or "hitchhikers"). Screening should be done either in the exporting country (preferably in quarantine conditions) just before shipment or in an intermediate country with an approved quarantine facility. Direct releases of natural enemies collected in the region of origin should be avoided. In the event that accidental contamination is discovered in either the exporting, intermediate or importing country, rapid communication between contracting parties should address the issue before further shipment occurs. Any plant materials accompanying the contaminated consignment, as well as the packaging, should be destroyed or sterilized, as per border quarantine operating procedures (if these are in place). Hyperparasitoids should be killed and sent for identification. As a quality assurance and validation measure, at least one generation of the BCA should be completed in containment (in as secure a facility as possible, given that quarantine

facilities are unavailable) before release. This will help ensure that BCA specimens are not carrying pests or diseases.

4.4 Rearing and multiplying biological control agents in quarantine

Imported BCAs should always be reared in quarantine through at least one generation to continue screening for contaminants (including hyperparasitoids).

In most cases, multiple generations will need to be reared to produce sufficient numbers of the BCA to test host specificity, for other studies (see section 4.5) and for release (if approval is granted). Usually, a BCA is reared on its target host species (i.e. the pest species); a population of the pest will be required, therefore, in the quarantine facility. Depending on the pest, it may be necessary to maintain live plant material for it to feed on. In this case, good practice is to grow the plant material in a quarantine greenhouse so that clean plants can regularly and securely be brought into the quarantine facility. In the absence of a greenhouse in the quarantine facility, an alternative secure greenhouse may be used, as long as adequate steps are taken to ensure no contaminating pests or diseases are brought into the quarantine facility.

In certain cases, an insect pest may be reared on an artificial diet. Such diets are feasible for wood-boring insects such as the emerald ash borer (*Agrilus planipennis*) and for defoliating moths such as the gypsy moth (*Lymantria dispar*). These diets may be modified to suit other insects with similar niches (e.g. other wood-borers and defoliators). Rearing an insect pest on artificial diets is easier than growing plant materials, but this option is not always available or suitable for BCAs.

Methods for rearing BCAs vary by species, and a basic understanding of a BCA's biology and its insect pest host is required for a successful rearing programme. Box 8 lists key biological characteristics that need to be understood for rearing an insect pest and its BCA.

BOX 8

Key biological characteristics to be understood when rearing an insect pest and its biological control agent

- Reproductive strategy: for example, does the insect reproduce sexually?
- Natural sex ratio
- Nutrient requirements at different life stages
- Fecundity
- Longevity of different life stages
- Temperature requirements
- Daylength requirements

Ideally, the necessary information for rearing a BCA and the target pest should be obtained before the BCA is imported. This may be available in published literature or

reports or from studies in the BCA's native range linked to the effort to introduce it. If no information on the BCA or pest can be obtained, biological studies will be needed in the quarantine facility. These should occur concurrently with efforts to multiply the BCA, with both activities dependent on the other.

4.5 Assessing the risk of introducing a biological control agent

The risks (likelihood and impact) associated with introducing a BCA to a new area should be fully understood before the BCA is released. For this, a risk assessment should be undertaken.

Growing awareness of the potential adverse effects of BCAs has prompted regulations and methods for risk assessment, the aim of which is to ensure that any non-native natural enemies introduced to an area are safe. A proper evaluation of the potential economic, social and environmental risks is now standard procedure.

The *Internet Book of Biological Control* (van Lenteren, 2012) describes the environmental risk-assessment process for introducing natural enemies. Some countries, such as Australia, New Zealand and the United States of America, have specific national regulations governing risk-assessment processes. When sufficient information is available, risk assessments may be conducted before a BCA is imported into quarantine.

The risk of introducing a BCA may be indirect or direct:

- *Indirect effects* are impacts on ecological communities or ecosystem processes through changes to the composition or structure of the food web (e.g. competition for food or space). For example, an introduced BCA may compete with native insects for the same resources (prey or host), resulting in a decline in native insect populations or the reduced suppression of the target pest. Although indirect effects should be considered, they can be difficult to test before the release of a BCA.
- *Direct effects* include impacts on non-target species, such as consumption, infection or parasitization by the BCA, and the hybridization of the BCA with a native species (which could affect the fitness, behaviour or ecology of the hybrid progeny). Introducing parthenogenetic biotypes is considered a solution to the issue of hybridization (Box 9).

BOX 9

Example of a parthenogenetic species used for classical biological control

In New Zealand, a "conditional release" was granted for the parasitoid *Microctonus aethiopoides* to control the weevil *Sitona lepidus*.

The conditional release allowed the importation of only the agent's parthenogenetic Irish biotype. This is because laboratory tests showed that other, non-parthenogenetic biotypes could hybridize with the Moroccan biotype (which had been introduced to control another *Sitona* species, *S. discoideus*). The hybrid offspring were inferior BCAs with the potential to compromise both classical biological control programmes.

The most commonly considered risk associated with introducing a BCA is the potential for the BCA to prey on or parasitize non-target species. Host-specificity tests are used to evaluate this risk.

Host-specificity and related studies

The aim of host-specificity studies is to identify potential non-target effects caused by introducing a BCA: in particular, they assess and predict whether a BCA will prey on or parasitize native (non-target) species or other species of concern and thereby reduce their populations. A list of non-target species should be developed for testing in consultation with taxonomists or experts in the target species' taxonomic group.

The study of other potential non-target effects is highly recommended. These might include:

- negative food-web effects (e.g. competition for prey, apparent competition, and displacing native species);
- positive food-web effects that benefit non-target species; and
- the potential for an introduced natural enemy to hybridize with native species.

Criteria exist for determining the non-target species that should be subject to testing (Box 10); other factors include the availability of such species and the potential for rearing them successfully in quarantine.

BOX 10

Criteria for selecting non-target test species

The selection for testing of non-target species in the area of intended introduction should be based on the following criteria:

- **Phylogenetically or taxonomically similar species.** These may be species in the same genus, subfamily or family of the target pest. In some cases, species in a closely related other family may be included.
- **Ecologically similar species.** These may be species that occupy a similar niche to the target pest species or have similar habits or characteristics, particularly if they are relevant to the biological control agent (BCA). If the intended BCA is a parasitoid of a gall wasp, for example, other gall-forming insects – especially those with similar gall morphology – could be considered as potential non-target species for testing.
- **Species of particular benefit or conservation importance.** These might include previously released BCAs or rare species with certain similarities (e.g. phylogenetic, taxonomic or ecological) to the target species.

Sometimes a potential non-target species cannot be reared in a laboratory and therefore cannot be tested in quarantine. In this case, permission may be requested from the NPPO or other relevant government authority to conduct semi-quarantine tests (e.g. within field cages) as an interim stage between importing it into quarantine and releasing it

into the environment. Because this approach increases the likelihood of a BCA escaping accidentally, however, strict risk-management measures must be put into place.

Methods for testing host specificity

Behavioural and developmental studies. Host-specificity studies assess the impacts of the behaviour of BCAs on non-target species. For a parasitoid BCA, for example, observations should include the presence or absence of oviposition behaviour towards the non-target species and whether the BCA can develop on the non-target species. The development of a BCA can be categorized into:

- incomplete development – in which BCA development continues only to a certain incomplete life stage; and
- complete development – in which a BCA completes its life cycle on the species.

For both behavioural and developmental studies, it is essential that the target species is included as a positive control to confirm that the testing method is sufficient. For example, if a BCA does not show positive behaviour towards the target host or does not complete its development on the target host, then the experimental design is flawed and should be reviewed. The conditions for studying different treatments, such as tests on non-target and target (control) species, must be standardized. This includes standardizing factors such as host age, mating and feeding history, and host biotype; for example, the same population of a BCA should be used for testing all the test species.

Choice studies. Both choice and no-choice studies can be used in host-specificity tests.

- Choice studies expose a BCA to a number of non-target species (and usually also the target species) simultaneously. Results indicate which species are preferred hosts.
- No-choice studies involve exposing a BCA to one host at a time to determine whether the BCA will develop on the host, even if it is not preferred.

Variations of choice and no-choice tests are available. No-choice tests should be performed first because, when these are negative, it can be assumed that choice tests are unnecessary.

Interpreting the results of host-specificity tests

If a BCA shows no interest in non-target species and does not develop on them, it can be considered host-specific. Note, however, that the degree of host specificity can never be stated with absolute certainty under laboratory or controlled conditions because it depends on the non-target species selected, which in turn is limited by current knowledge and the availability of species for testing. Nevertheless, host-specificity studies should provide good evidence of the likely specificity or non-specificity of an intended BCA.

If a BCA develops completely or partially on one or more of the non-target species tested, or shows interest (for example, oviposition) in any of the non-target species, further consideration and experimentation may be required. A case can still be argued for host specificity if a BCA attacks a non-target species but does not complete development; further experiments are advisable, however, to confirm the lack of full development.

When there is complete development on a non-target species, the suitability of that BCA should be reconsidered. Decisions on the suitability for release of a BCA should also take other factors into account, such as the:

- preference of the BCA for the non-target species;
- likely impact of the BCA on that species; and
- likelihood of the BCA encountering that species in the field.

These factors will require investigation.

The methods used for host-specificity tests should be planned carefully and documented clearly.

As well as studies on a proposed BCA's host specificity and biology, studies should be undertaken to determine whether the BCA is likely to have an impact on the pest population. In most cases, such studies assess the levels of parasitism or predation achieved by a BCA. Although study results may not accurately reflect parasitism or predation levels in the field, they can provide an indication of the likely effectiveness of a BCA (see Chapter 5).

Occasionally, non-target effects can have unexpected benefits, such as when an introduced BCA also attacks a non-target non-native pest species (Box 11).

BOX 11

Host-specificity study of native and non-native species as non-targets

Biological control agent: *Psyllaephagus bliteus*
Intended host: *Glycaspis brimblecombei*
Additional biological control agent host: *Spondyliaspis* c.f. *plicatuloides*, a non-native, recently introduced insect and a pest of *Eucalyptus* spp.

A host-specificity study of the parasitoid *Psyllaephagus bliteus* (a biological control agent of *Glycaspis brimblecombei*) in South Africa showed that a non-native species, *Spondyliaspis* c.f. *plicatuloides*, was also a host of the BCA. *S.* c.f. *plicatuloides*, however, is an introduced pest of *Eucalyptus* and is not the BCA's preferred host (compared with *Glycaspis brimblecombei*) and therefore the benefit of releasing the BCA outweighs the risk. (In this case, release was not required anyway because the BCA had already been introduced to the country unintentionally.)

4.6 Releasing biological control agents from quarantine

Releasing a BCA from quarantine is a major decision. In a CBC programme, the research and study phases, as well as importing a BCA into quarantine, can all be stopped at any time without significant impact. Releasing a BCA from quarantine, however, is the point of no return.

Applying for release

An application to release is made to the NPPO or other relevant government authority when the results of host-specificity and other studies (see section 4.5) indicate that the

intended BCA is suitable for release. When a direct release is planned, it may be possible to obtain import and release authorization in a single application. BCA release-approval applications are not standardized. To help the application, as much relevant information as possible should be provided about the BCA release. National policies and relevant documentation should be consulted in preparing the release application. Suggested topics to include are as follows:

- a brief background of the target insect pest, including its impact and any control options available;
- the value of, and need for, the proposed BCA;
- a description of the proposed BCA, including taxonomy, biology, origin, recorded host species, and any regions in which the BCA has previously been released;
- results of host-specificity and related studies – these will include any known possible indirect ecological effects of the proposed BCA;
- data and discussion on the efficacy of the proposed BCA against the target pest;
- a cost–benefit analysis comparing current or potential losses (economic, social and environmental) from the pest with the expected benefits of releasing the BCA;
- in the case of expected non-target effects, a discussion of the possible environmental impacts and a benefit–risk assessment; and
- the proposed release strategy, including:
  - the number and location of the intended release sites
  - for insect BCAs, the number of individuals to be released at each site
  - the frequency of releases (whether a single release or multiple releases)
  - the organizations and facilities involved in rearing the BCA
  - the time required to rear the BCA (predefined or long-term)
  - any need for further BCA imports.

Data on pest population levels

The release strategy should include baseline data on the pest population as it is before the initial release ("pre-release survey"). This enables comparisons of pest population levels before and after release. The method used for collecting pest population data in before-release and after-release surveys, preferably at the same sites, should be consistent.

Confirming identity of the biological control agent before release

A BCA's identity to species level should be reconfirmed before release, and voucher or reference specimens should be retained and deposited with at least two key national organizations. Reference specimens can be used to ensure that any future introductions in the same or other areas are of the same species (see section 4.3). Potentially, reference specimens can also be used to determine whether different lineages of the same BCA species have been released. Where possible, reference specimens should be stored in a freezer in 96 percent ethanol for potential future comparisons of DNA sequence data.

Releasing biological control agents into the field environment

When a BCA has already been released in a different area, details of the release method may be available. Otherwise, the process for release can be developed by referring to previous releases of similar BCAs and/or by conducting field trials. The "propagule pressure" – that is, the number of BCA individuals (for insect BCAs) released per site and the number of release sites – can differ between species and areas because establishment depends on the interaction between the founding population and the environment. It is important, therefore, to document all information pertaining to a release and to consider such information in planning post-release monitoring (see Chapter 5).

Regulatory requirements

Before the initial BCA release, an official authorization to perform field releases must be obtained from the NPPO or other relevant authority.

Communication strategy

A clear communication strategy is essential for informing and engaging stakeholders potentially affected by a release, including farmers, producer organizations, forest companies, researchers, universities, neighbouring countries and the public. The NPPO or other authority has primary responsibility for communicating with all relevant parties on a BCA release. Releasing BCAs can be highly contentious, and it is important for stakeholders to support the communication efforts of the NPPO or other authority as much as possible.

Selecting sites for release of biological control agents

The selection of an initial release site should take into consideration the BCA's preferred habitat and the density of the target pest. Potential sites should have easy, unrestricted access, and they should have no recent (or planned) intensive pest-control activities.

Initial release sites should be used for validation experiments, such as:

- evaluating a BCA's parasitism or predatory capacity in field conditions;
- climatic or weather effects during and after release;
- BCA establishment and spread; and
- tree recovery.

To facilitate such assessments, the exact release points should be marked (e.g. with stakes) and the coordinates recorded using a global positioning system device.

Newly released BCAs are likely to have been reared in laboratory conditions in a quarantine facility and may be especially sensitive to unfavourable weather in the field-release environment. The release should be timed to ensure, as much as possible, that optimum weather conditions apply. In many cases, failure to establish a BCA in a region is related directly to previous applications of chemical insecticides. It is important, therefore, to select release sites that have not recently been sprayed with insecticides or other toxic chemicals.

Methods for releasing biological control agents

There are various methods for releasing BCAs in the field. The method selected should be matched to the type of natural enemy and its life cycle and dispersal mechanism and the developmental stage at which it is collected and shipped. It is important that the phenology of a BCA is synchronized with that of the pest when deciding on the timing and method of release.

Leptocybe invasa parasitoid *Selitrichodes neseri* prepared for release

Conditions during release. Ideally, BCAs should be kept cool (but not cold) and shaded during transportation, with adequate ventilation in containers to avoid condensation. Survival rates can be improved by providing food sources (e.g. pollen or nectar for parasitoids) during transportation and at the release site. In general, BCAs should be released when temperatures are moderate (e.g. in the morning or late afternoon/early evening on hot days and at midday on cool days); insect BCAs should not be released on rainy days.

Insect BCAs. The simplest way to release insect BCA larvae, nymphs or adults into the field environment is to tap an opened container onto a pest-infested host plant. The BCAs should fall out or emerge and seek out the pest. Adults will disperse, so one release point (e.g. a single plant) per site is often sufficient. On the other hand, actively feeding immature stages are less mobile and should be scattered on several plants to ensure they can find sufficient food.

Selitrichodes neseri is released on a tree

Containment of released *S. neseri*

Pathogen BCAs. For fungal pathogens in particular, the preferred release methods are to:

- spray or dust several pest-host plants with a suspension of spores in water; or
- place presprayed plants in pots at the release site.

In both cases, the pathogen's humidity requirements should be met for the first 6–8 hours. This means that either release should occur on a misty or rainy day, or the treated plants should be misted and covered with plastic bags overnight to maintain humidity.

Spores will wind-disperse and travel long distances from the initial point of release.

Release methods for pathogenic nematodes differ from those for fungal pathogens; see case study 7.8 for the release method used for BCAs of the sirex woodwasp.

Aerial application. When a target area is large or the terrain difficult, BCAs may be released aerially. Planes and helicopters used in conventional aerial pest-control operations are being replaced by drones for this purpose because they are more flexible and cheaper to operate.

Post-release establishment. To enable BCA populations to establish and grow, attention should be paid to the release site for a certain period after release to ensure that:

- the site is not sprayed with insecticide;
- plants are not destroyed; and
- plants or plant parts that support the BCA's life cycle are not removed.

Use the data collected and lessons learned from an initial release to increase the effectiveness of subsequent releases in other areas. Steps should be taken to ensure that sufficient BCAs are reared for wider release areas.

Rearing procedures for further releases of biological control agents

Researchers should develop protocols for rearing BCAs in quarantine. For field releases, these protocols should be adapted to reproduce a given BCA on a larger scale outside the quarantine area once permission to release has been granted. If possible, BCAs for release should be reproduced in insectaries inside greenhouses or screened cages. Alternatively, it might be possible to recover BCAs directly from post-release field sites and to transfer them to new release sites.

A bucket funnel trap for catching cerambycid beetles

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5 Monitoring and evaluating classical biological control

5.1 Post-release monitoring

Monitoring BCA performance during pre-release, release and post-release is a crucial final step in implementing a CBC programme. Accurate monitoring will ascertain the effectiveness and impacts of a BCA, enable ongoing comparisons between regions, and help improve future programmes.

A post-release monitoring system includes:

- sampling methods for the BCA and target pest;
- monitoring procedures to regularly observe, measure and record the BCA's populations, dynamics, weather, host-tree conditions (e.g. pest damage symptoms), pest populations, infestation levels and potential competitors or natural enemies of the BCA; and
- assessing non-target effects of the BCA.

In developing sampling methods, it is useful to identify population benchmarks for both the BCA and the target pest. Such benchmarks can be described using the following parameters of population expression:

- *population density* – the number of individuals per available food resource (e.g. host or prey insects for BCAs and leaves, buds, seeds, fruit or biomass for herbivorous insects);
- *relative population* – the number of individuals per sampling unit (e.g. tree canopy or trap) – samples might be collected using knockdown insecticides (in a small area or tree canopy) or traps (e.g. light traps and sex or aggregation pheromone traps); and
- *population indexes* – indirect measures of insect density such as frass weight, damage severity and percent defoliation.

Population density and relative population are the best parameters for BCA population expression, and population density can be used to estimate parasitism (Box 12) and predation. Relative population is useful when a BCA cannot be sampled directly from its host, for example when the tree canopy is very high.

Before setting traps to capture BCAs, previous studies should be consulted to evaluate the best trap designs and tree position(s). Malaise traps and coloured sticky traps are suitable for many insect parasitoids.

Population indexes can be used to assess pest populations when it is difficult to apply other sampling methods. These are easier and less time-consuming to use, although they require more time for verifying differences in pest symptoms and signs. In most cases, population indexes are unreliable for correlating a reduction in pest population with

A sticky trap used to monitor the establishment of *Selitrichodes neseri*

true BCA effectiveness, but they are useful for evaluating defoliation (e.g. by *Gonipterus* weevils) and gall severity (e.g. gall-wasp infestations).

Once the sampling methods have been decided, monitoring procedures should be determined for the selected sites. Monitoring data can be used to understand the dynamics of a pest population and the climatic and seasonal factors that affect it. To ensure consistent results, the same monitoring procedures should be used at all sites where a pest has been identified. Assessing data from ongoing monitoring efforts ensures the accurate measurement of BCA effectiveness, pest reductions and non-target effects.

Effectiveness of biological control agents

Assessing the effectiveness of a BCA can start in the early stages of introduction to help indicate the likelihood of ongoing success. Key assessment parameters are:

- Establishment
- Population growth
- Dispersal.

These parameters are assessed by monitoring BCA numbers:

- over time – to assess establishment and population growth; and
- within and beyond the introduced area – to assess dispersal.

Results will help determine whether the level of establishment, population growth and dispersal of a BCA is likely to suppress the target pest to below the desired economic or environmental threshold.

Biological control agent establishment. The failure of a BCA to establish may be due to reasons unassociated with the BCA's suitability. Releasing a low number of individuals is a common reason for failing to achieve establishment. Other causes influencing the survival of the initially released populations include competition with other insects; predation and parasitism; unsuitable release timing; and severe weather events following release. If the first population is too small, either because too few individuals were released or because of high mortality after release, the population may go extinct due to the "allee effect" – which occurs when the population is very small or highly dispersed in its new environment, reducing the likelihood that individuals will encounter the opposite sex, mate and reproduce.

In many cases, the factors affecting BCA establishment can be overcome by changing the:

- size of each release to find the optimal release size;
- number of releases (frequency);
- release season; or
- release method (for example, releasing different developmental stages).

Note that, in rare cases, BCA populations have established successfully despite their release in small numbers (ten or fewer individuals).

Biological control agent population growth. Established populations of a BCA may be difficult to detect at first, particularly when survey procedures are inadequate (e.g. sample sizes are too small). There may be a time lag between a field release and recording the presence of a BCA, with some BCAs remaining at low and sometimes undetectable densities for several generations after release. This is referred to as the "eclipse period" (also "lag phase"), when populations stay small while the BCA adapts to local conditions. Although populations of some BCAs grow very fast, others will require several years to reach population sizes capable of having an impact on a target pest. The number of generations produced per year will influence the speed at which populations grow.

Biological control agent population dispersal. High dispersal ability is a desirable attribute of BCAs because it enables faster control of a pest population and reduces the number of release points needed. A high dispersal ability can, however, also reduce the abundance of a BCA, both at the release site and at non-release sites, due to migration and an "area dilution" effect. In this case, population density will decline towards the margins of the geographical range of expansion because individuals spread out over a progressively larger area.

A BCA with low dispersal ability will require greater effort to ensure that it spreads throughout the introduced range, likely increasing the cost of the CBC programme. This, however, should not be a reason for dismissing the BCA from the programme.

BOX 12

Assessing biological control agent effectiveness: parasitism rates

Objective assessments of the effectiveness of released parasitoids require accurate estimates of parasitism rates. These can be made by applying the "rearing" and "dissection" methods to:

- field-collected host (pest) insects; or
- plant material if the host species feeds in concealed or semi-concealed situations (e.g. leaf miners, gallmakers and borers).

The rearing method is a count of parasitoid adults (and their host) emerging from the host or from material previously exposed to parasitism.

The dissection method is an estimate of the proportion of a host attacked by parasitoids made by dissecting infested hosts or material, often under a stereomicroscope.

The rearing method is easy to perform but may provide a poor estimate of parasitism rates because the host and its parasitoid may show different survival rates in laboratory rearing. Moreover, rearing may continue for a long period, and insect emergence might be delayed by many factors (e.g. diapause or weather conditions). This is problematic if parasitism must be confirmed quickly, for example to make prompt decisions about further releases.

On the other hand, dissection provides fast and often more-accurate estimates of parasitism rates, but there are associated difficulties. For example, parasitoid eggs or larvae may be difficult to find in or on a host; moreover, if a host is attacked by a cohort of closely-related parasitoids, it is not always easy to identify the species using the morphology of immature stages. In such cases, it may necessary to use PCR [polymerase chain reaction]-based molecular techniques.

For maximum accuracy, it is best to use both the rearing and dissection methods concurrently.

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A dissected gall of the Asian chestnut gall wasp (*Dryocosmus kuriphilus*, see case study 7.3). The dissection was performed to detect the number of parasitoids (in this case pupae of *Torymus sinensis*) in the larval chambers. Parasitoids usually develop from a single host larva so that the number of killed hosts is equal to the number of parasitoids found

Pest reduction

Pre-release surveys, combined with pest monitoring, will confirm whether pest numbers have declined, but this reduction may not be due to the BCA alone. Year-to-year pest abundance can vary substantially due to climatic fluctuations, land-use change, tree susceptibility, forest practices and the impacts of native natural enemies. To make objective assessments of the impacts of BCAs, standardized quantitative surveys need to be repeated over time.

Temporal comparisons. It is desirable to collect pre-release data for pest damage and the effectiveness of native natural enemies to enable temporal ("before and after") comparisons for assessing the effectiveness of a BCA release. For example, a CBC programme in Italy was implemented against the Asian chestnut gall wasp (case study 7.3) that had caused a significant (up to 80 percent) decrease in chestnut yields. Monitoring of native parasitoid efficacy at different sites showed that, 3–5 years after the arrival of the pest, the average rate of parasitism was very low (0.45 percent). A few years after the release of the BCA *Torymus sinensis*, its efficacy was estimated by considering chestnut production recovery (at least 50 percent of the values observed before the pest's arrival) and the high mortality inflicted on the pest by both the BCA (up to 90 percent) and native parasitoids (2 percent).

Spatial comparisons. A pest reduction caused by a BCA can be confirmed by comparing areas where the BCA is already present with areas where it is absent (spatial "with and without" comparisons). This is particularly useful when pre-release data are insufficient for carrying out a temporal comparison, for example when serious damage caused by an invasive species requires urgent action.

Both spatial and temporal comparisons should ideally be carried out to assess pest-reduction rates and the impact of a released BCA.

BOX 13

Non-target effects of the biological control agent *Compsilura concinnata*

Insect pest: Gypsy moth, *Lymantria dispar*

Biological control agent: *Compsilura concinnata*

Important non-target effects were assessed for *Compsilura concinnata*, a tachinid parasitoid introduced to North America at the beginning of the twentieth century to control an invasive forest pest, the gypsy moth (*Lymantria dispar*). This biological control agent is a broad, generalist species with more than 200 known hosts. It achieved significant parasitism (up to 80 percent) and contributed to the decline of several native moth populations in the area of introduction.

The introduction of *C. concinnata* in North America also had a beneficial effect by unintentionally controlling the brown-tail moth, *Euproctis chrysorrhoea*, an invasive pest. This positive effect does not lessen its impact on native biodiversity, however, and such polyphagous species should not be considered for classical biological control.

Non-target effects

As stressed in section 4.5, it is important to determine whether a BCA introduction will have a positive, negative or neutral impact on local ecosystems. After release, there is an opportunity to confirm the validity of pre-release risk assessments and tests on host-range impacts. For example, a post-release monitoring study was carried out in North America on the BCA *Peristenus digoneutis*, which is an egg parasitoid of *Lygus* plant bugs. The study revealed that the impact of the parasitoid on non-target species was significantly less than predicted by laboratory analysis. Nevertheless, BCA releases can have negative non-target impacts (Box 13).

5.2 Evaluating the success of a classical biological control programme

The success of a CBC programme is determined by the extent to which it meets the objective of safely reducing the impacts of pests on trees and the environment. The reduction of pest numbers to an acceptable threshold, and limited non-target effects, would be indicators of a successful CBC programme. The extent of success can be better understood by answering the following three questions:

1. To what extent has the BCA been effective, and why?
2. To what extent was the pest reduction caused by the BCA or other factors?
3. What was the extent of any non-target effects?

It must be noted that even partial success (e.g. a reduction in pest density that remains permanently or occasionally above a threshold level) may have a positive effect if it reduces the use of costly or unsustainable pest-control methods.

Another aspect to consider in determining the success of a CBC programme is how well it has been implemented. Analysing the implementation of each step of the programme will provide a greater understanding of the extent of its success and help in identifying ways to improve future programmes. Questions about a CBC programme's implementation might include:

- Has the programme gone according to plan?
- How effectively and efficiently was each step in the programme carried out, including the accuracy of assessments and studies undertaken, the communication strategy and other social considerations?
- Has stakeholder engagement been appropriate?
- Has the cost of the programme kept within the allocated budget?

Assessing the impact of a classical biological control programme

In addition to assessing the success of a CBC programme, its wider impact should also be determined, including its economic, social and environmental benefits. Relevant questions include:

- Has the CBC programme resulted in increased or restored forest production?
- Has the pest reduction led to a reduction in insecticide use?
- Have jobs been created or protected?

Estimating all the benefits of a CBC programme will increase the credentials of future programme proposals.

Megarhyssa nortoni, *a parasitoid wasp of the Sirex woodwasp,* Sirex noctilio

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6 Other considerations

6.1 The Nagoya Protocol

The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization, a supplementary agreement to the Convention on Biological Diversity (CBD), came into force on 12 October 2014. The CBD recognizes that countries have sovereign rights over genetic resources within their boundaries and that CBD parties have the authority to determine access rights to these genetic resources. The Nagoya Protocol provides a framework for the fair and equitable sharing of benefits arising from the use of genetic resources, including BCAs used in CBC programmes against forest insect pests.

CBC involves the release of living organisms into new environments. BCAs that become established, reproduce and spread will have a self-sustaining effect on target pests. Such BCAs thus become "common goods" available to all, and CBC programmes must comply with regulations governing access and benefit-sharing arising from the Nagoya Protocol.

Countries that have signed up to the Nagoya Protocol are required to develop and introduce frameworks to ensure shared access to genetic resources, benefit-sharing and compliance. Many signatory countries allow free access as part of their frameworks, but some place restrictions on the import or export of genetic resources, including BCAs. These restrictions can hinder the successful implementation of CBC in managing transboundary pests.

FAO, the CBD, the Centre for Agriculture and Bioscience International and the International Organisation for Biological Control (IOBC) work to promote the use of BCAs under special considerations of the Nagoya Protocol and to facilitate compliance with the Protocol. The IOBC provides guidelines for best practices that maintain continued, legitimate access to BCAs for research and use as a public good.

6.2 Stakeholder engagement

The importance of the ongoing involvement of, and meaningful communication with, all stakeholders throughout the course of a CBC programme cannot be overstated.

In many countries, stakeholder engagement on the implementation of CBC is conducted by NPPOs or other relevant national organizations. Very early in the process (even before a BCA is imported into quarantine) it is beneficial to develop a communication plan jointly with the NPPO or other relevant organization and to identify all the affected stakeholders, including the public.

Good stakeholder communication is very important in forestry, where large, highly biodiverse areas are often subject to pest management. A lack of communication can

result in misconceptions about the impacts of a CBC programme, and miscommunication can lead to barriers to the use of CBC as an option in IPM.

Compared with other pest-control methods, the benefits of CBC as a self-sustainable, long-term pest-management option are often less apparent and underestimated. Also, the risk-assessment process for introducing BCAs must be transparent. At the beginning of a CBC programme, the process should be explained clearly to all stakeholders, including forest practitioners and managers, non-governmental organizations and the public, through training workshops, farmer field schools and the media and by distributing fact sheets about the programme. A participatory approach is the best option for implementing CBC programmes.

6.3 The way forward

As shown in this guide, implementing an effective CBC programme is a multistep process requiring a highly coordinated approach by a wide range of actors and organizations, including regulators, scientists, forest managers and practitioners. Many countries that would benefit from effective CBC programmes lack access to the required knowledge and resources. Ongoing collaboration and information-sharing between countries, as well as regionally and globally, is vital, therefore, for ensuring successful CBC outcomes. Stakeholder consultation and participation is also essential: CBC works best when it is inclusive and championed by many.

Several national and regional organizations and networks, including working groups of the IOBC, promote CBC as part of IPM and actively encourage the sharing of information and resources. Such organizations also encourage participation, and membership is open to representatives of countries and organizations as well as to individuals. The knowledge gained, support given and assistance provided is invaluable for preventing, detecting and managing forest insect pest outbreaks.

In looking ahead, it is important to consider the role that all stakeholders can play in challenging negative perceptions of CBC, which may exist due to the adverse environmental impacts of past CBC programmes. It is crucial to ensure that future CBC programmes are based on sound scientific data and analysis and best management practices and to communicate this to the public. There is a need to promote CBC and its successes widely to ensure its greater acceptance among scientists, forest practitioners, the public and decision-makers. This, in turn, will help ensure the safe and effective deployment of CBC in the world's forests.

Release of the parasitoid
Torymus sinensis

© F. COLOMBARI

7 Case studies

7.1 Coconut rhinoceros beetle in the Pacific region

Insect pest: *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae)
Biological control agent: *Oryctes rhinoceros nudivirus* (OrNV)

Male (top) and female (bottom) coconut rhinoceros beetles

Insect pest

The coconut rhinoceros beetle (*Oryctes rhinoceros* – CRB), a native of Southeast Asia, is a major invasive pest of coconut and oil-palm trees in the Pacific region. The adult beetle bores into the crowns of palms, where it damages growing tissue and feeds on tree sap, cutting across young fronds and flowers. This reduces leaf area, causes early nut fall, decreases yields and creates open wounds that can become infected by decay organisms. The damage significantly reduces production and can lead to tree death.

Biological control programme

CRB has been managed successfully in the Pacific since the 1960s using a viral BCA, *Oryctes rhinoceros nudivirus* (OrNV), as part of IPM. OrNV was discovered in Malaysia in the nuclei of CRB larval fat-body cells and the nuclei of midgut epithelium cells (of larvae and adults), as well as in the adult ovarian sheath and spermatheca. The virus is transmitted orally and also deposited in feeding sites through beetle faeces. Once infected, larvae die within 1–4 weeks. The virus did not exist in any South Pacific country before its introduction as a BCA in the 1960s.

Impact of classical biological control

This is an example of the highly successful, practical use of an insect virus in CBC, and it is a landmark case. The virus has established successfully at many sites in Fiji, Mauritius, Papua New Guinea, Tokelau and Tonga and on the Samoan islands and Wallis Island. It significantly reduces beetle damage to palms within 1.5 years of establishment.

After 40 years, some Pacific Islands with established CRB populations – such as Guam, Hawaii, Palau, Papua New Guinea and Solomon Islands – reported an increased severity and frequency of damage caused by CRB. Attempts to introduce OrNV into the Guam CRB population have been unsuccessful, and it is possible that the CRB population biotype on Guam is tolerant of or resistant to OrNV isolates. Efforts are underway to identify a virus in CRB's native range that will be effective against the newly described biotype CRB-G.

Key lessons

- The use of a virus in a CBC programme can cause persistent pest suppression over time.
- To achieve a virus's optimal control capacity, it is essential to comply with IPM principles.
- To maintain high levels of control, additional biotypes of a BCA may be required over time or for different populations of the pest.

Further reading

Huger (2005); Jackson (2009).

7.2 Great spruce bark beetle in Europe

Insect pest: *Dendroctonus micans* Kugelann (Coleoptera: Curculionidae, Scolytinae)
Biological control agent: *Rhizophagus grandis* Gyllenhal (Coleoptera: Rhizophagidae)

Insect pest

The great spruce bark beetle (*Dendroctonus micans*) lives under the bark of spruce trees (*Picea* spp.) and is sometimes found in other conifers. It is probably native to northern Siberia, but it has spread westward over the past 100 years. *D. micans* was first noticed in Belgium in 1897 and in the United Kingdom of Great Britain and Northern Ireland in 1982, where it caused significant mortality to sitka spruce (*P. sitchensis*). The beetle is still expanding its range, although it has not yet reached Ireland or Spain. From the Russian Federation it has also spread southward: it was first recorded in Georgia in 1957, from where it entered Turkey. Under endemic conditions, the beetle is widespread but causes only minor damage, and outbreaks occur mainly on the edges of its expanding range, where heavy tree losses due to repeated attacks have been observed.

Biological control programme

The first biological control programme was developed in the Georgian Soviet Socialist Republic in the 1960s, a few years after the pest was detected in the region and where it caused severe damage to oriental spruce (*P. orientalis*). A predatory beetle, *Rhizophagus grandis*, specific to *D. micans*, was known to control bark beetle populations in central

An adult *Rhizophagus grandis*

Europe. Adults of *R. grandis* are attracted by volatiles in frass produced by *D. micans* and lay eggs among the prey's brood. *R. grandis* larvae and adults feed on the eggs and larvae of *D. micans* in their galleries. This predator, imported from Czechoslovakia, was released in Georgia as a BCA, with a series of rearing units set up to further distribute the species. A similar CBC programme was later implemented in Turkey.

CBC programmes to combat *D. micans* were set up in central and western France and the United Kingdom of Great Britain and Northern Ireland in the 1980s, and hundreds of thousands of *R. grandis* specimens were produced and released. With *D. micans* continuing to spread in both countries, *R. grandis* is still being released regularly in newly infested areas.

Impact of classical biological control

Rhizophagus grandis has established quickly in all areas in which it has been introduced, and pest outbreaks have usually collapsed within 6–10 years. In France and the United Kingdom of Great Britain and Northern Ireland, there is a strong link between reduced pest populations and the increased predator levels encountered in the main infested areas.

Key lessons

- Natural enemies do not always closely follow their host or prey when a pest is invading new areas. In such cases, CBC is appropriate to assist natural enemies in their spread and to restore natural control.
- Although many predators are generalists and thus not suitable for CBC, some are very specific to their prey and can be used successfully as BCAs.

Further reading

Fielding and Evans (1997); Grégoire (1988).

Larvae of *Rhizophagus grandis* feeding on *Dendroctonus micans* larva

7.3 Asian chestnut gall wasp in North America, northern Asia and Europe

Insect pest: *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae)
Biological control agent: *Torymus sinensis* Kamijo (Hymenoptera: Torymidae)

Insect pest

The Asian chestnut gall wasp (*Dryocosmus kuriphilus*) (ACGW) is a specialist gallmaker on chestnuts (all *Castanea* spp. and their hybrids) native to China. After its accidental introduction to Japan, the Republic of Korea, North America and Nepal (in the 1940s, 1950s, 1970s and 1990s, respectively), the ACGW arrived in northwestern Italy in 2002 and spread rapidly throughout Europe. Parthenogenetic females emerge in summer and lay eggs in newly developed chestnut buds, where the larvae overwinter. In spring, attacks by the ACGW cause galls to form on growing leaves and shoots, potentially damaging tree growth and reducing fruit, wood and honey production. Moreover, trees weakened by intensive ACGW attacks are likely to be highly susceptible to other disturbances, such as drought and infection by pathogens.

Biological control programme

Native parasitoids in invaded countries have been unable to control the ACGW, due largely to mismatches in life cycles. To control the ACGW, therefore, extensive CBC programmes have been implemented in Japan, the United States of America and, more recently, Italy to introduce *Torymus sinensis*, a parasitoid specific to the ACGW in China. *T. sinensis* has one generation per year, like its host, and its flight period coincides with the appearance of the host galls, where it lays eggs. Larvae of *T. sinensis* develop within the galls, killing the pest larvae before overwintering and pupation. Releases in Italy usually comprised 150 individuals with a female-to-male ratio of 2:1. This propagule size was sufficient to establish the parasitoid successfully at most sites.

Impact of classical biological control

Declines in ACGW populations to below threshold levels of damage were reported in Japan and the United States of America following the introduction of *T. sinensis* as a BCA – indirect evidence of the parasitoid's efficacy and its ability to expand its geographic range alongside expanding ACGW populations. In northwestern Italy, parasitism as high as 85 percent was recorded eight years after release of the BCA. In Veneto in northeastern Italy, chestnut stands are distributed over an area of 20 500 hectares, and severe losses in both nut production (down by 80 percent) and wood yield (down by 40 percent) were reported before BCA release. Almost 500 BCA releases were made over six years. The BCA reached non-release sites (via short- and long-distance flights), up to 70 km away from the nearest release site, within 3–5 years of first release. The average mortality inflicted on the pest was 82.5 percent, resulting in a recovery in yield of at least 50 percent. Recent studies have shown that *T. sinensis* has had minimal impact on

A chestnut branch heavily infested by the Asian chestnut gall wasp. The large reduction in leaf area results in a decline in photosynthetic capacity, affecting tree growth

non-target cynipid wasps, and it is able to follow fluctuations in the host population in both time and space.

Key lessons

- Specific parasitoids that are well synchronized with their hosts can have a large impact on pest populations.
- BCAs are able to respond to the spatial distribution of hosts, ensuring the rapid colonization of the host's geographic range.
- Habitat structure (e.g. resource concentration, such as host-containing patches and food) can play a fundamental role in the rapid establishment, growth and spread of introduced BCAs.

Further reading

Battisti *et al.* (2014); Colombari and Battisti (2016a); Colombari and Battisti (2016b); Ferracini *et al.* (2017); Colombari and Battisti (2017).

7.4 Orthezia bug on Saint Helena

Insect pest: *Insignorthezia insignis* Browne (Hemiptera: Ortheziidae); syn: *Orthezia insignis* Browne (Hemiptera: Ortheziidae)
Biological control agent: *Hyperaspis pantherina* Fürsch (Coleoptera: Coccinellidae)

Insect pest

The orthezia bug (*Insignorthezia insignis*) is a polyphagous scale insect from South and Central America that is now widespread throughout the tropics. It has been controlled successfully in Hawaii, several African countries and Peru using its specific predator, the coccinellid beetle (*Hyperaspis pantherina*). In 1991, the orthezia bug was found to have severely affected the endemic and highly valued gumwood tree (*Commidendrum robustum*) on the small South Atlantic island of Saint Helena. At the time of this first record, the gumwood population was restricted to 2 000 individuals. Two years later, 400 of the 2 000 had been killed.

Biological control programme

In collaboration with international organizations, the Government of Saint Helena, Ascension and Tristan da Cunha initiated a CBC programme immediately after the

The orthezia bug, *Insignorthezia insignis*

damage was discovered. The *H. pantherina* beetle was collected in Kenya, where it successfully controls the same scale on other trees. The collected specimens were imported to the United Kingdom of Great Britain and Northern Ireland for quarantine studies, which showed that the predator lives and develops in close association with the orthezia bug and is probably specific to it. Because no closely related indigenous scales are present on Saint Helena Island, releasing the coccinellid beetle there was considered safe for the environment.

Impact of classical biological control

The introduction of *H. pantherina* on Saint Helena resulted in the rapid collapse of the orthezia bug population. Within two years, mean orthezia bug numbers per 20-cm branchlet had decreased from more than 400 adults and nymphs to fewer than 15. Outbreaks of the scale have not been observed since 1995, and *H. pantherina* is considered to have averted gumwood's extinction. This is probably the first CBC programme implemented for the purpose of saving a tree from extinction.

Key lessons

- As well as being effective for commercial crops, CBC can be a valuable management method for protecting the environment against invasive pests.
- Fast reaction is essential to save trees endangered by an invasive pest.
- Previous CBC successes can serve as the basis for planned new CBC programmes.

Further reading

Cock *et al.* (2015); Fowler (2004).

Orthezia bug infestation on gumwood in St Helena

7.5 Eucalypt gall wasp

Insect pest: *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae)
Biological control agents: *Quadrastichus mendeli* Kim & La Salle (Hymenoptera: Eulophidae); *Selitrichodes kryceri* Kim & La Salle (Hymenoptera: Eulophidae); *Megastigmus zvimendeli* Doganlar & Hassan (Hymenoptera: Torymidae); *Megastigmus lawsoni* Doganlar & Hassan (Hymenoptera: Torymidae); *Selitrichodes neseri* Kelly & La Salle (Hymenoptera: Eulophidae)

Insect pest

Leptocybe invasa is a small wasp (1–1.5 mm in length) of presumed Australian origin that induces galls on the leaf midribs, petioles and stems of susceptible *Eucalyptus* species. It was first detected in the Middle East and Mediterranean in 2000, and within ten years it had spread to all regions of the world in which eucalypts are grown. Damage can range from the complete write-off of plantations at establishment (caused by heavy seedling malformation) to negative impacts on growth rates in young trees.

L. invasa mainly reproduces parthenogenetically, and males are found only rarely. This ability to reproduce asexually has contributed significantly to the species' success as a highly invasive pest. The spread of the wasp has also been assisted by the movement

A female *Selitrichodes neseri*, a biological control agent for the eucalypt gall wasp

of infested nursery stock around the world. Another – currently undescribed – species of *Leptocybe* is also spreading; in many areas it co-occurs with *L. invasa*. The two species are morphologically indistinguishable and can only be identified with molecular barcoding.

Biological control programme

Israeli scientists carried out surveys of *Leptocybe*-like galls in eastern Australia in 2006, collecting four parasitoids to use in Israel's CBC programme – two eulophid wasp species, *Quadrastichus mendeli* and *Selitrichodes kryceri*, and two torymid wasp species, *Megastigmus zvimendeli* and *M. lawsoni*. These four parasitoids were released in Israel in 2007. A South African scientist discovered a fifth parasitoid, *Selitrichodes neseri*, in Australia in 2010, and this was released in South Africa in 2012 and Brazil in 2015 and then in a number of other countries in Africa and South America.

Impact of classical biological control

All four parasitoids released in Israel became established and are now exerting substantial control over *L. invasa* there and in most of the Mediterranean region. *Quadrastichus mendeli* and *M. zvimendeli* appear to be the most successful and abundant of the four parasitoids, but all four are helping reduce population densities of *L. invasa* in Israel. Initial data show that *S. neseri* has established successfully in South Africa and Brazil, and its establishment in other countries is being assessed. *Q. mendeli* has been recorded moving unassisted to several parts of the world, including Africa and Southeast Asia. In some locations, apparently native parasitoids have switched to *L. invasa* as a host and are contributing significantly to the control of pest populations.

All parasitoids so far released against *L. invasa* have been collected on another, undescribed species of *Leptocybe* in Australia (*L. invasa* is yet to be found in Australia). The short- and long-term impacts of this on the biocontrol of both *Leptocybe* species is unknown.

Key lesson

Introducing and releasing multiple parasitoids against an invasive pest may achieve higher and more stable levels of control under a variety of climatic conditions and range of tree hosts. Single parasitoid species, often sourced from a single location in a pest's endemic range, are less able to adapt to different climates and may also have lower genetic variability.

Further reading

Dittrich-Schröder *et al.* (2018); Mendel *et al.* (2004); Mendel *et al.* (2017).

7.6 Bronze bug on *Eucalyptus* in Brazil

Insect pest: *Thaumastocoris peregrinus* Carpintero & Dellapé (Hemiptera: Thaumastocoridae)
Biological control agent: *Cleruchoides noackae* Lin & Huber (Hymenoptera: Mymaridae)

Insect pest

The bronze bug (*Thaumastocoris peregrinus*), which is native to Australia, has become a serious pest of eucalypt plantations in Africa, South America and Europe. It was introduced unintentionally to South Africa in 2003, Argentina in 2005 and Italy in 2011, and it spread quickly across Africa, South America and Europe, causing silvering and

A female *Cleruchoides noackae* parasitizing a bronze-bug egg

bronzing in eucalypt canopies and intense defoliation. The bronze bug can infest almost any commercially planted *Eucalyptus* species and clone. Data from forest inventories of eucalypt plantations in different parts of Brazil over the period 2010–2015 show a reduction in wood production of 10–20 percent and estimated losses of USD 330 million.

Biological control programme

The egg parasitoid *Cleruchoides noackae* was introduced to Brazil from Australia in 2012, with the first releases in infested plantations in the states of São Paulo and Minas Gerais. Females of *C. noackae* parasitize *T. peregrinus* eggs and adults emerge from the egg operculum, making it difficult to evaluate effective parasitism. The life cycle (egg to adult) of *C. noackae* spans about 16 days at 24 °C, and the adult stage spans 1.1–3.6 days. A new egg-laying technique using paper towels enabled a higher production of pest eggs and parasitoids and, by 2018, the parasitoid had established in four Brazilian states, with more than 250 000 parasitoids reared and released in the country in 2014–2016. Laboratory and field evaluations have shown parasitism rates of 50–60 percent, reaching as high as 90 percent in some places. The parasitoid was introduced to Uruguay in 2015, successfully reducing pest populations in eucalypt plantations there, too.

Impact of classical biological control

The CBC programme successfully reared, released and established the parasitoid in plantations in five areas. Three years after the first releases of *C. noackae*, the infested area had been reduced by 18.8 percent (compared with 2012). In addition, avoiding the use of chemical insecticides enabled native natural enemies, such as the predatory bugs *Atopozelus opsimus* and *Supputius cincticeps*, to assist in reducing pest populations. The CBC programme will continue until the parasitoid has been established in all Brazilian states.

Key lessons

- An egg parasitoid with a lifespan synchronized with its host and well adapted to field conditions can be highly effective in reducing pest populations.
- Enhancing rearing techniques improves the production of BCAs and reduces the time before parasitoids become established and control is effective.
- By allowing a reduction in the use of chemical insecticides, the introduction of successful BCAs helps native natural enemies play complementary roles in pest management.

Further reading

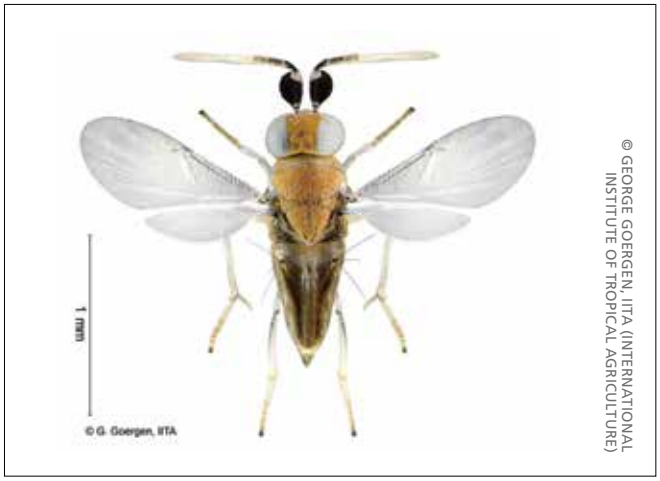
Barbosa *et al.* (2017); Dias *et al.* (2014); Mutitu *et al.* (2013); Soliman *et al.* (2012); Wilcken *et al.* (2010).

7.7 Mango mealybug in West Africa

Insect pest: *Rastrococcus invadens* Williams (Hemiptera: Pseudococcidae)
Biological control agents: *Gyranusoidea tebygi* Noyes (Hymenoptera: Encyrtidae); *Anagyrus mangicola* Noyes (Hymenoptera: Encyrtidae)

Insect pest

Mango (*Mangifera indica*) is a highly valued fruit and shade tree in West Africa. The mango mealybug (*Rastrococcus invadens*), which originates in Southeast Asia, was first found in Ghana and Togo in 1981. Nymphs and adults affect plant leaves, inflorescences and fruit by sucking the sap, but the main damage is due to the honeydew secreted by the bug. This secretion allows a black sooty mould to develop, which limits photosynthesis and reduces tree growth and fruit production. In Benin, yield losses due to the mango mealybug were estimated at 89 percent. The pest is polyphagous, and attacks have also been observed on other fruits, such as banana and citrus, although mango appears to be preferred.



Anagyrus mangicola, parasitoid of the mango mealybug

Biological control programme

Benin's national authorities developed a CBC programme in 1986 in collaboration with various international organizations. Surveys for natural enemies were carried out in India and Malaysia, and two abundant and previously undescribed parasitoids found in India were sent to the United Kingdom of Great Britain and Northern Ireland for quarantine studies. These parasitoids, later described as *Gyranusoidea tebygi* and *Anagyrus mangicola*, occupy different ecological niches: *G. tebygi* attacks young nymphs and *A. mangicola* targets older females. Moreover, laboratory tests showed that the parasitoids are specific to the mango mealybug and it was decided therefore to introduce the two species. Releases started in 1987 for *G. tebygi* and in 1991 for *A. mangicola*. The two species

were later distributed in many countries throughout West and Central Africa from a mass-rearing facility in Benin. They also spread naturally to other African countries and are now present in the entire area occupied by the mango mealybug.

Individuals of the mango mealybug, *Rastrococcus invadens*

Impact of classical biological control

The results of the CBC programme were spectacular, with mealybug populations dropping significantly throughout West and Central Africa. In Benin, mango production increased by 142 percent. The CBC programme lasted nine years and cost USD 3.66 million, and the benefits were huge. Cost–benefit ratios of 1:145 and 1:808 were calculated for Benin and sub-Saharan Africa, respectively, to 1999 and are likely much higher today. The calculation of the ratios did not take into account the non-monetary value of mango trees for shade and medicine and as a source of energy and vital nutrients for rural communities.

Key lessons

- Two effective parasitoids can be introduced together when they occupy different ecological niches.
- Huge benefits can be achieved with CBC because they persist over time without further action.

Further reading

Cock *et al.* (2015); Moore (2004).

7.8 Sirex woodwasp in Australasia, Africa, the Americas and Asia

Insect pest: *Sirex noctilio* Fabricus (Hymenoptera: Siricidae)
Biological control agents: *Deladenus* (Beddingia) *siricidicola* Bedding (Tylenchida: Neotylenchidae); *Ibalia leucospoides* Hochenwarth (Hymenoptera: Ibalidae); *Megarhyssa nortoni* Cresson (Hymenoptera: Ichneumonidae); *Rhyssa persuasoria persuasoria L.* (Hymenoptera: Ichneumonidae)

Insect pest

The sirex woodwasp (*Sirex noctilio*) is native to Europe, North Africa and parts of Asia and has been introduced unintentionally to most pine-growing areas of Australasia, Africa, the Americas and Asia. The sirex woodwasp has become a serious pest of *Pinus* species when the trees are grown as non-natives in plantations. The wasp introduces a symbiotic fungus (*Amylostereum areolatum*) and a toxic mucous into pines during oviposition, resulting in the eventual death of the trees.

Biological control programme

The first report of the sirex woodwasp outside its native range was in New Zealand in about 1900; it was reported in Tasmania, Australia, in 1952 and on the Australian mainland in 1961. Increasing infestations of the sirex woodwasp, particularly in Australia,

Inoculation of nematodes into a sirex woodwasp-infested tree

prompted a programme in the 1960s and 1970s to search for BCAs, both in its native range and in North America, where other *Sirex* species occur. This programme tested a number of natural enemies as potential BCAs.

A parasitic nematode (*Deladenus siricidicola*) and a number of parasitic wasps were released into the field environment in the invaded range (after considering host specificity, recorded prevalence – effectiveness – on the target host, and suitability for mass-rearing).

D. siricidicola has a bicyclic life cycle. It parasitizes larvae of the sirex woodwasp in the parasitic life cycle and remains in its host until pupation, when it moves to the reproductive organs and infests and sterilizes the female's eggs. The female wasp oviposits the sterile eggs into host trees, which helps spread the nematode. In the fungus-feeding life cycle, *D. siricidicola* feeds on *A. areolatum* (the symbiotic fungus of the sirex woodwasp). The nematode's life cycle on the fungus lasts only about two weeks, which means large numbers of the nematode can be reared in the laboratory on fungal cultures in a short period. Mass-reared nematodes are dispatched to the infested areas in breathable plastic bags, with water. Polyacrylamide powder is then added to the nematode–water mixture, forming a gel that is inoculated into holes made in infested trees with specialized inoculation hammers.

Of the parasitic wasps introduced as BCAs against the sirex woodwasp, the most successful have been *Ibalia leucospoides*, *Megarhyssa nortoni* and *Rhyssa persuasoria*. The first of these parasitizes the eggs and early larval instars of the woodwasp and the other two parasitize its mature larvae.

Impact of classical biological control

The use of CBC to manage populations of the sirex woodwasp is considered to have been largely successful where it has been applied in Australasia, Africa and South America. In the late 1980s, it was reported that the strain of nematode released previously had lost virulence, possibly because the fungal-feeding form had been cultured for more than 20 years. This prompted the selection of a new nematode strain for release.

The extent of parasitism achieved by the parasitic wasp BCAs is reported to vary between regions with differing climates, and the nematode control method has consequently been applied in some of those areas.

Key lessons

- The ability to mass-rear and effectively apply the parasitic nematode *D. siricidicola* to infested trees, together with the high rates of parasitism obtained in many areas, contributed to the use of this nematode as the primary BCA of the sirex woodwasp.
- BCAs may lose virulence over time and are not always effective uniformly between areas. It may be necessary, therefore, to release new strains and to adapt local CBC programmes over time.

Further reading

Bedding and Iede (2005); Hurley, Slippers and Wingfield (2007); Slippers, Hurley and Wingfield (2015).

7.9 Winter moth in Canada and the United States of America

Insect pest: *Operophtera brumata L.* (Lepidoptera: Geometridae)
Biological control agents: *Cyzenis albicans* Fallén (Diptera: Tachinidae); *Agrypon flaveolatum* Gravenhorst (Hymenoptera: Ichneumonidae)

Insect pest

The winter moth (*Operophtera brumata*) is native to Europe, where it is considered a sporadic pest of various deciduous trees. It was introduced unintentionally to Nova Scotia in eastern Canada in the first half of the twentieth century and quickly became a serious pest of forest trees there, particularly oak (*Quercus*) species but also orchard trees such as cherry and apple. In the 1950s, red oak (*Q. rubra*) in Nova Scotia suffered a mortality rate as high as 40 percent due to *O. brumata* outbreaks; later, the moth appeared on the west coast of Canada. In the United States of America, *O. brumata* appeared first on the west coast and, more recently (in the 1990s), it was reported in Massachusetts on the east coast. Caterpillars of the winter moth defoliate trees in spring, and the flightless females emerge from pupae in the soil in late autumn or early winter to lay eggs that hatch just before bud burst, when they start feeding on fresh leaves.

Larva of the winter moth, *Operophtera brumata*

Biological control programme

Six parasitoids were introduced to Nova Scotia from Europe in the 1950s. Two became established: a tachinid fly, *Cyzenis albicans*, and an ichneumonid wasp, *Agrypon flaveolatum*. Both species parasitize moth larvae and overwinter in pupa in the soil, but they have different roles in the moth's population dynamics. *Cyzenis albicans* can reduce moth populations at outbreak densities, and *A. flaveolatum* probably plays a more important role at relatively low host densities. It has been shown that the establishment of the two parasitoids in eastern Canada favoured moth predation by some key local generalist predators that also help keep pest populations at low densities. *Cyzenis albicans* is specific to the winter moth, but there is uncertainty about the host range of *A. flaveolatum*, largely due to its uncertain taxonomic status.

After the success of the CBC programme in eastern Canada in the 1950s, the two parasitoids were released in western Canada (British Columbia) and western United States of America (Oregon) in the early 1980s, where they quickly became established, causing outbreaks to collapse. In Massachusetts, only *C. albicans* was released because it is highly specific to the winter moth (*A. flaveolatum* can also attack closely related moth species), and it has been established in that state since 2012.

Impact of classical biological control

The introduction of the two parasitoids to Canada is a classic case of successful CBC against a forest pest. The winter moth is now only a very minor pest in eastern Canada, where it causes less damage than in its area of origin. Populations of the moth also declined rapidly on the North American west coast following the establishment of *C. albicans* and *A. flaveolatum*, although, in some areas, densities are higher than in Nova Scotia. In Massachusetts, ongoing studies suggest that *C. albicans* alone will control the pest.

Key lessons

- Two natural enemies that play different roles in the population dynamics of a pest can be complementary in a CBC programme.
- Introduced natural enemies may enhance the effectiveness of native natural enemies on an invasive pest.
- Resolving the taxonomy of a natural enemy is essential for assessing its suitability as a potential BCA.

Further reading

Elkinton and Boettner (2014).

7.10 Ambermarked birch leaf miner in Canada and the United States of America

Insect pest: *Profenusa thomsoni* Konow (Hymenoptera: Tenthedinidae)
Native congeneric: *Profenusa canadensis* Marl. (Hymenoptera: Tenthedinidae)
Biological control agents: *Lathrolestes thomsoni* Reshchikov (Hymenoptera: Ichneumonidae); *Aptesis segnis* Provancher (Hymenoptera: Ichneumonidae); *Lathrolestes soperi* Reshchikov (Hymenoptera: Ichneumonidae)

Insect pest

The ambermarked birch leaf miner (*Profenusa thomsoni*) is a rare species in its native range of mainly peat bogs in central and northern Europe. Its larvae mine the leaves of birch trees (*Betula* spp.) but never cause significant damage. The species was introduced accidentally to North America at the beginning of the twentieth century, and it became a serious pest of birch in Alberta and the Northwest Territories, Canada, in the 1970s and in Alaska, United States of America, in the 1990s. Damage was particularly high in urban trees, with up to 90 percent of leaves damaged, severely affecting the aesthetic value of the trees.

Larval mines caused by the ambermarked birch leaf miner (*Profenusa thomsoni*)

Biological control programme

A CBC programme was initiated in the early 1990s to control *P. thomsoni* in Alberta. Surveys carried out in Europe found parasitoids that could potentially be introduced to Canada. In 1994, however, populations of *P. thomsoni* in Alberta crashed unexpectedly due to parasitism from a previously unknown ichneumonid wasp, *Lathrolestes thomsoni* (then designated as *L. luteolator*), an internal parasitoid of larvae in leaf miners.

L. thomsoni was also observed controlling the leaf miner in the Northwest Territories. It was introduced intentionally as a BCA in Alaska in 2004–2009 and became established at all release sites.

L. thomsoni has never been found on *P. thomsoni* in Europe, so it probably came from another host species in North America, although the possibility that it was introduced accidentally from Europe with its host, or from another region, cannot be ruled out. Interestingly, *L. thomsoni* was recently found parasitizing *P. thomsoni* in Massachusetts, United States of America, where the leaf miner has never occurred at outbreak densities since its arrival at the beginning of the twentieth century; it is possible that the parasitoid has been present in that region for a long time.

Impact of classical biological control

Populations of *P. thomsoni* in Alberta crashed within two years of the arrival of *L. thomsoni* and have remained low since. Damage levels also decreased significantly in Alaska, although in that region the decline of *P. thomsoni* was also attributed at least partly to two other ichneumonid parasitoids, *Aptesis segnis* and *L. soperi. A. segnis* is a native ectoparasitoid, attacking last instar host larvae in their earthen cells in soils – this species is also known from a native congeneric leaf miner, *Profenusa canadensis*. *L. soperi* is a larval endoparasite which, like *L. thomsoni*, is of unknown origin and has never been found on another host.

Key lesson

Invasive insects can be accidentally controlled by native natural enemies that attack the invader and by non-native natural enemies that arrive unintentionally.

Further reading

Digweed *et al.* (2003); Soper, MacQuarrie and Van Driesche (2015).

7.11 Fall webworm in China

Insect pest: *Hyphantria cunea* Drury (Lepidoptera: Arctiidae)
Biological control agent: *Chouioia cunea* Yang (Hymenoptera: Eulophidae)

Insect pest

The fall webworm (*Hyphantria cunea*), a native of North America, is an invasive forest insect pest in China. It was discovered there in 1979 in Dandong, a town in Liaoning Province on the border with the Democratic People's Republic of Korea. The fall webworm has since spread to many other parts of China, including the provinces of Anhui, Hebei, Shaanxi and Shandong and the municipalities of Beijing and Tianjin.

The fall webworm defoliates many species of ornamental, forest and fruit trees as well as agricultural crops (it is known to use 175 plant species as hosts in 49 families and 108 genera). Initially, pesticides were applied in an attempt to control the species; due to the side-effects of pesticide use, however, biological control was explored in conjunction with other measures as a sustainable alternative for containing further spread and damage.

Larvae of the fall webworm larvae (*Hyphantria cunea*), showing the leaf damage they can cause

Biological control programme

A search was conducted in China for effective native insect natural enemies of the fall webworm; previous attempts to control the species in Europe using insect parasitoids introduced from North America had only limited success. Surveys conducted in the early 1980s in Shaanxi Province discovered potential native predators and parasitoids. Subsequent surveys in 1996–2000 throughout the range of the fall webworm in China resulted in the identification of 27 potential insect predators and parasitoids. Among these, an endoparasitic chalcid wasp, *Chouioia cunea*, which parasitizes the pupae of the fall webworm, was identified as an effective BCA. A mass-rearing and augmentative release programme was developed for the chalcid wasp using an alternative host, *Antheraea pernyi.*

Impact of classical biological control

Chouioia cunea has been deployed widely as an effective BCA as part of an IPM approach for the fall webworm in China.

Further reading

Yang (1989); Yang (1990); Yang *et al.* (2008).

The boxwood tree moth, *Cydalima perspectalis*, on a boxwood tree

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Glossary

The definitions provided in this glossary have been collected from publications and the Internet and edited for clarity; note that not all terms listed here are used in the main text of this publication. Although the definitions of terms used in forestry and many other technical fields are often highly variable, considerable effort has been made to find common understandings to reduce confusion. In forestry, FAO and the International Union of Forest Research Organizations have collaborated for many years on this issue, producing, for example, a glossary on forest genetic resources. FAO has also developed terms and definitions for its Global Forest Resources Assessments, taking into account the recommendations of experts in various fora. Except in a few instances, this glossary provides only one definition for each term (usually with the source of the definition indicated), but readers should be aware that other meanings may exist. Note that the ISPMs use the definitions specified in ISPM 5 exclusively. For the latest definitions of the International Plant Protection Convention (IPPC), please refer to its website (www.ippc.int). For the latest definitions of the IOBC, please refer to its website (www.iobc/global.org).

Allee effect	A reduction in the growth rate of a species at low densities (Jang and Diamond, 2007)
Area	An officially defined country, part of a country, or all or parts of several countries (ISPM 5)
Augmentation biological control	A biological control method that involves mass or repeated releases of BCAs
Augmentative release	Either inundation or seasonal inoculative release: i.e. forms of biological control that release mass-produced BCAs to reduce a pest population without necessarily leading to continued impact or establishment (van Lenteren, 2012)
Beneficial organism	An organism directly or indirectly advantageous to plants or plant products, including BCAs (van Lenteren, 2012)
Biological control	A pest management strategy making use of living natural enemies, antagonists or competitors and other self-replicating biotic entities (van Lenteren, 2012)
Biological control agent	A natural enemy, antagonist or competitor, or another organism, used for pest control (ISPM 5)
Biopesticide	Generally applied to a BCA, usually a pathogen, formulated and applied in a manner similar to a chemical pesticide and normally used for the rapid reduction of a pest population for short-term pest control (ISPM 3)
Biotype	A group of organisms having the same genotype
Classical biological control	The intentional introduction and permanent establishment of an exotic biological agent for long-term pest management (van Lenteren, 2012)
Competitor	An organism that competes with pests for essential resources (e.g. food, shelter) in the environment (van Lenteren, 2012)
Congeneric	Animal or plant species belonging to the same genus

Conservation control	A biological control method that conserves existing natural enemies in a system by providing them with conditions under which they can thrive
Consignment	A quantity of plants, plant products and/or other articles being moved from one country to another and covered, when required, by a single phytosanitary certificate (a consignment may be composed of one or more commodities or lots) (ISPM 5)
Contaminant (in the introduction of invertebrate BCAs)	Any unwanted organism or substance included in the commerce of natural enemies that poses a risk to the health of natural enemies, humans and/or ecosystems (van Lenteren, 2012)
Cryptic species	One or more morphologically indistinguishable species that are incapable of interbreeding
Delimiting survey	A survey conducted to establish the boundaries of an area considered to be infested by or free from a pest (ISPM 5)
Direct effect (from the introduction of an exotic biocontrol agent)	A physical interaction between a BCA and target or non-target organisms (effects can be positive, negative or neutral) (van Lenteren, 2012)
Eclipse period (or lag phase)	A period during which the abundance of an introduced BCA may fall below a detection threshold, possibly due to post-colonization adaptations of the introduced species to local conditions in a variable environment
Ecological host range	The range of species that a natural enemy parasitizes/feeds on/infects in nature [see also *physiological (= fundamental) host range*] (van Lenteren, 2012)
Efficacy (of a biological control agent)	The ability to cause a statistically significant reduction in the number of pest organisms or the extent of direct and indirect crop damage or yield losses (van Lenteren, 2012)
Entomophagous	Pertaining to organisms that eat insects (van Lenteren, 2012)
Entomopathogens	Pathogens of insects
Environmental risk assessment	An analysis of the likelihood of occurrence and the magnitude of consequences of adverse environmental effects (van Lenteren, 2012)
Eradication	The application of phytosanitary measures to eliminate a pest from an area (ISPM 5)
Establishment (of a species)	The successful long-term survival and reproduction of a species after introduction into a new area (van Lenteren, 2012)
Establishment (of a pest)	The perpetuation, for the foreseeable future, of a pest in an area after entry (ISPM 5)
Establishment (of a biological control agent)	The perpetuation, for the foreseeable future, of a BCA in an area after entry (van Lenteren, 2012)
Exotic	Not native to a particular country, ecosystem or ecoregion (area) (van Lenteren, 2012)
Fecundity	The number of eggs a female of a species can produce during her lifetime (Frank and Gillett-Kaufman, 2018)
Forest	1) Land spanning more than 0.5 hectares, with trees higher than 5 metres and a canopy cover of more than 10 percent, or trees able to reach these thresholds *in situ.* It does not include land that is predominantly under agricultural or urban land use (FAO, 2007); 2) a biological community of plants and animals dominated by trees and other woody plants (Hubbard, Latt and Long, 1998)
Forestry	The science of establishing, cultivating and managing forests and their attendant resources (Hubbard, Latt and Long, 1998)
Generalist	See "host specificity"

Genetic diversity	The genetic variability within a population or a species. It is one aspect of biological diversity. Genetic diversity can be assessed at three levels: a) diversity within breeding populations; b) diversity between breeding populations; and c) diversity within the species (FAO/IUFRO, 2002)
Genotype	The genetic constitution of an organism, as distinguished from its appearance or phenotype (FAO/IUFRO, 2002)
Germplasm	Plants intended for use in breeding or conservation programmes (FAO, 1990)
Habitat	Part of an ecosystem with conditions in which an organism naturally occurs or can establish (ISPM 5)
Host range	1) The suite of host species capable, under natural conditions, of sustaining a specific pest or other organism (ISPM 5); 2) the set of species that allows survival and reproduction of a natural enemy [see also *physiological (= fundamental) host range* and *ecological host range*] (van Lenteren, 2012)
Host specificity	A measure of the host range of a BCA on a scale ranging from "extreme specialist" (where the BCA only completes development on a single species or strain of its host – monophagous), to "generalist" (where the BCA has many hosts, including several groups of organisms – polyphagous) (van Lenteren, 2012)
Host-specific	A parasite, parasitoid or pathogen that, at least in the area specified, is monophagous (Frank and Gillett-Kaufman, 2018)
Hyperparasitism (also *hyperparasitoidism*)	The parasitism (parasitoidism) of a parasitoid. A second-arriving parasitoid attacks the first and becomes a hyperparasitoid of the host (Frank and Gillett-Kaufman, 2018)
Hyperparasitoid (also *hyperparasite*)	A parasitoid that uses another parasitoid as a host (van Lenteren, 2012)
Import permit	An official document authorizing the importation of a commodity in accordance with specified phytosanitary import requirements (ISPM 5)
Import permit (for a BCA)	An official document authorizing importation of a BCA in accordance with specified requirements (van Lenteren, 2012)
Inbreeding	The mating of genetically related individuals (van Lenteren, 2012)
Indigenous species	Species or genotypes that have evolved in the same area, region or biotope and are adapted to those specific, predominant ecological conditions. Species native to the country or area. Antonym: non-native or exotic (FAO, 1994) (see also *native species*)
Indirect effect (from the introduction of a BCA)	The effect that the introduction of a BCA has on other organisms that does not involve physical interaction with the BCA (effects can be positive, negative or neutral) (van Lenteren, 2012)
Infestation (of a commodity)	The presence in a commodity of a living pest of the plant or plant product concerned. Infestation includes infection (ISPM 5)
Inspection	The official visual examination of plants, plant products or other regulated articles to determine whether pests are present and/or to determine compliance with phytosanitary regulations (ISPM 5)
Integrated pest management	A pest population management system that uses all suitable techniques in a compatible manner to reduce pest populations and maintain them at levels below those causing economic injury (Smith and Reynolds, 1966; FAO has adopted this definition)
Interbreeding	Breeding between different species (van Lenteren, 2012)
Introduced (species)	1) A species, subspecies or lower taxon [of trees] occurring outside its natural range (past or present) and dispersal potential (i.e. outside the range it occupies naturally or could occupy without direct or indirect introduction or care by humans) (FAO, 2007); 2) an established species not native to the ecosystem, region or country (FAO/IUFRO, 2002)

Introduction (of a pest)	The entry of a pest resulting in its establishment (ISPM 5)
Introduction (of a biological control agent)	The release of a BCA into an ecosystem where it did not exist previously (van Lenteren, 2012)
Invasive species	Species that are not native to a particular ecosystem and whose introduction and spread cause, or are likely to cause, sociocultural, economic or environmental harm, or harm to human health (FAO, 2007)
Inundative/inundation release	The release of very large numbers of a mass-produced BCA with the expectation of achieving a rapid reduction of a pest population, without necessarily achieving continued impact or the establishment of the BCA (van Lenteren, 2012)
Insect biological control agent	See *invertebrate biological control agent*
Invertebrate biological control agent	An invertebrate natural enemy used for pest management (van Lenteren, 2012)
Kairomone	A chemical produced by one insect species that influences the behaviour of another species
Lag phase	See *eclipse period*
Legislation	Any act, law, regulation or other administrative order promulgated by a government (van Lenteren, 2012)
Likelihood (in risk assessment)	A qualitative description of probability (van Lenteren, 2012)
Magnitude (in risk assessment)	A qualitative descriptor of the size of the consequences if adverse or beneficial effects occur (see also *risk*) (van Lenteren, 2012)
Magnitude of risk of establishment	The area within which an introduced natural enemy is potentially able to establish as a percentage of the area in which the exotic natural enemy will be licensed (e.g. a whole country or part of it) (van Lenteren, 2012)
Management or control of a pest	The suppression, containment or eradication of a pest population (van Lenteren, 2012)
Microbial control	The use of microorganisms (including viruses) as BCAs (van Lenteren, 2012)
Microorganism	A protozoan, fungus, bacterium, virus or other microscopic self-replicating biotic entity (van Lenteren, 2012)
Monitoring	An official ongoing process to verify phytosanitary situations (ISPM 5)
Monophagous	An organism that attacks only one host species and is species-specific (van Lenteren, 2012)
Mutualism	An association between organisms of two species in which both species benefit (van Lenteren, 2012)
National plant protection organization	An official service established by a government to discharge the functions specified by the IPPC (ISPM 5)
Native	Naturally occurring in an area of proposed BCA release (van Lenteren, 2012)
Native species	A species, subspecies or lower taxon occurring within its natural range (past or present) and dispersal potential (i.e. within the range it occupies naturally or could occupy without direct or indirect introduction or care by humans) (IUCN, 2000) (see also *indigenous species*)
Natural enemy	An organism that lives at the expense of another organism in its area of origin and which may help to limit the population of that organism. This includes parasitoids, parasites, predators, phytophagous organisms and pathogens (ISPM 5)

Naturally occurring	Refers to a component of an ecosystem or a selection from a wild population not altered by artificial means (van Lenteren, 2012)
Non-target organisms	All organisms except the target organism (van Lenteren, 2012)
Nudivirus	A large, rod-shaped virus with circular, double-stranded DNA
Oligophagous	An organism that attacks a limited group of related hosts (e.g. up to 20 species in the same genus or subfamily) (van Lenteren, 2012)
Organism	A biotic entity capable of reproduction or replication; includes vertebrate and invertebrate animals, plants and microorganisms (van Lenteren, 2012)
Outbreak	A recently detected pest population, including an incursion, or a sudden significant increase of an established pest population in an area (ISPM 5)
Parasite	An organism that lives on or in a larger organism, feeding on it (van Lenteren, 2012)
Parasitoid	An insect that is parasitic only in its immature stages, kills its host in the process of its development, and is free-living as an adult (ISPM 5)
Parthenogenetic	A type of asexual reproduction whereby the development of eggs occurs without fertilization
Pathogen	A microorganism causing disease (van Lenteren, 2012)
Pathway	Any means that allows the entry or spread of a pest (ISPM 5)
PCR-based molecular techniques	Techniques used in molecular biology to amplify a single copy or a few copies of a segment of DNA
Pest	Any species, strain or biotype of a plant, animal or pathogenic agent injurious to plants or plant products (ISPM 5)
Pest risk (for quarantine pests)	The probability of introduction and spread of a pest and the magnitude of the associated potential economic consequences (ISPM 5)
Pest risk analysis	The process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated, and the strength of any phytosanitary measures to be taken against it (ISPM 5)
Pest risk management (for quarantine pests)	The evaluation and selection of options to reduce the risk of introduction and spread of a pest (ISPM 5)
Pheromone	A chemical produced by a species that affects the behaviour of other insects of the same species
Physiological (= fundamental) host range	The range of species that a natural enemy can parasitize/feed on/infect in the laboratory (see also *ecological host range*) (van Lenteren, 2012)
Phytosanitary certificate	An official document issued by an NPPO to indicate that a consignment of plants, plant products or other regulated articles meets specified phytosanitary import requirements and is in conformity with the requirements of the NPPO of the importing country. Such certificates are patterned after the model certificates of the IPPC (ISPM 5)
Phytosanitary certification	The use of phytosanitary procedures leading to the issuance of a phytosanitary certificate (ISPM 5)
Phytosanitary import requirements	Specific phytosanitary measures established by an importing country concerning consignments of plants, plant products or other regulated articles moving into that country (ISPM 5)
Phytosanitary measure	Any legislation, regulation or official procedure having the purpose of preventing the introduction or spread of quarantine pests or limiting the economic impact of regulated non-quarantine pests (ISPM 5)
Phytosanitary security (of a consignment)	The maintenance of the integrity of a consignment of plants, plant products or other regulated articles and the prevention of its infestation and contamination by regulated pests through the application of appropriate phytosanitary measures (ISPM 5)

Planted forest	A forest predominantly composed of trees established through planting or deliberate seeding (FAO, 2007)
Plants	Living plants and parts thereof, including seeds and germplasm (ISPM 5)
Polyphagous	An organism that attacks a wide range of hosts in different subfamilies (van Lenteren, 2012)
Predator	A natural enemy that preys and feeds on other animal organisms, more than one of which are killed during its lifetime (van Lenteren, 2012)
Prey	A living organism that serves as food for a predator (Frank and Gillett-Kaufman, 2018)
Propagule size (release size)	the number of individuals in each introduction event (Lockwood, Cassey and Blackburn, 2005)
Propagule pressure (also *introduction effort*)	A composite measure of the number of individuals released into a region to which they are not native. Incorporates estimates of the absolute number of individuals involved in any one release event (propagule size) and the number of discrete release events (propagule number)
Quarantine (of a biological control agent)	The official confinement of a BCA subject to phytosanitary regulations for observation and research or for further inspection or testing (van Lenteren, 2012)
Release (into the environment)	The intentional liberation of an organism into the environment (van Lenteren, 2012)
Release (of a consignment)	Authorization for entry after clearance (van Lenteren, 2012; ISPM 5)
Risk	The combination of the likelihood of occurrence and the magnitude of consequences should the effects occur (van Lenteren, 2012)
Risk management options	Risk reduction actions that may be selected, alone or in combination, to reduce identified risks to an acceptable level (= risk mitigation) (van Lenteren, 2012)
Risk mitigation	see *risk management options*
Semiochemical	A chemical substance or mixture that carries a message for the purpose of communication within or between species
Sentinel plant	A living plant cultivated and moved to a location that enables the detection of native or exotic pests and diseases that may attack these species
Silviculture	The growing and cultivating of trees
Soft insecticides	Insecticides with few off-target impacts
Specialist	See *host specificity*
Species	A population or series of populations of organisms that are capable of interbreeding freely with each other but not with members of other species (FAO/IUFRO, 2002) (see also *indigenous species*, *introduced species* and *native species*)
Spread	The expansion of the geographical distribution of a pest in an area (ISPM 5)
Suppression	The application of phytosanitary measures in an infested area to reduce pest populations (van Lenteren, 2012)
Surveillance	An official process for collecting and recording data on pest occurrence or absence by survey, monitoring or other procedures (ISPM 5)
Survey	An official procedure conducted over a defined period to determine the characteristics of a pest population or which species occur in an area (ISPM 5)
Treatment	The official procedure for the killing, inactivation or removal of pests, for rendering pests infertile, or for the devitalization of pests (ISPM 5)
Uncertainty	The estimated amount by which an observed value may differ from the true value due to incomplete or wrong information (van Lenteren, 2012)

The larva of a boxwood tree moth, Cydalima perspectalis, *on a boxwood tree*

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Annex: List of pests, biological control agents and tree hosts mentioned in the guide

Species	Authority	Taxonomic order	Common name	Role
Agrilus planipennis	Fairmaire	Coleoptera: Buprestidae	Emerald ash borer	Pest
Agrypon flaveolatum	Gravenhorst	Hymenoptera: Ichneumonidae		Biological control agent; parasitoid
Amylostereum areolatum	(Fr.) Boid			Fungus symbiont
Anagyrus mangicola	Noyes	Hymenoptera: Encyrtidae		Biological control agent; parasitoid
Antheraea pernyi	Guerin-Meneville	Lepidoptera: Saturniidae		Alternative host
Aptesis segnis	Provancher	Hymenoptera: Ichneumonidae		Biological control agent; ectoparasitoid
Betula spp.	L.	Fagales: Betulaceae	Birch	Tree host
Buxus spp.	L.	Buxales: Buxaceae	Boxwood	Tree host
Castanea spp.	Mill.	Fagales: Fagaceae	Chestnut	Tree host
Chouioia cunea	Yang	Hymenoptera: Eulophidae		Biological control agent; endoparasite
Cleruchoides noackae	Lin & Huber	Hymenoptera: Mymaridae		Biological control agent; parasitoid
Cocos nucifera	L.	Arecales: Arecaceae	Coconut	Tree host
Compsilura concinnata	Meigen	Diptera: Tachinidae		Biological control agent; parasitoid
Commidendrum robustum	(Roxb.) DC.	Asterales: Asterceae	Gumwood	Tree host
Cydalima perspectalis	Walker	Lepidoptera: Crambidae	Boxwood tree moth	Pest
Cyzenis albicans	Fallén	Diptera: Tachinidae		Biological control agent; parasitoid
Deladenus (Beddingia) *siricidicola*	Bedding	Tylenchida: Neotylenchidae		Biological control agent; parasitic nematode
Dendroctonus micans	Kugelann	Coleoptera: Curculionidae, Scolytinae	Great spruce bark beetle	Pest

Species	Authority	Taxonomic order	Common name	Role
Dryocosmus kuriphilus	Yasumatsu	Hymenoptera: Cynipidae	Asian chestnut gall wasp	Pest
Elaeis guineensis	Jacq.	Arecales: Arecaceae	Oil palm	Tree host
Eucalyptus spp.	L'Hér	Myrtales: Myrtaceae	Eucalypt	Tree host
Euproctis chrysorrhoea	L.	Lepidoptera: Lymantriidae	Brown-tail moth	Pest
Glycaspis brimblecombei	Moore	Hemiptera: Aphalaridae	Red gum lerp psyllid	Pest
Gonipterus spp.	-	Coleoptera: Curculionidae	Eucalyptus weevils	Pest
Gyranusoidea tebygi	Noyes	Hymenoptera: Encyrtidae		Biological control agent; parasitoid
Hyperaspis pantherina	Fürsch	Coleoptera: Coccinellidae	Coccinellid beetle	Biological control agent; predator
Hyphantria cunea	Drury	Lepidoptera: Arctiidae	Fall webworm	Pest
Ibalia leucospoides	Hochenwarth	Hymenoptera: Ibalidae		Biological control agent; parasite
Icerya purchasi	Mask.	Homoptera: Margarodidae	Cottony cushion scale	Pest
Insignorthezia insignis	Browne	Hemiptera: Ortheziidae	Orthezia bug	Pest
Lathrolestes luteolator	Gravenhorst	Hymenoptera: Ichneumonidae		Biological control agent; parasitoid
Lathrolestes soperi	Reshchikov	Hymenoptera: Ichneumonidae		Biological control agent; endoparasite
Lathrolestes thomsoni	Reshchikov	Hymenoptera: Ichneumonidae		Biological control agent; parasitoid
Leptocybe invasa	Fisher & La Salle	Hymenoptera: Eulophidae	Eucalyptus gall wasp	Pest
Lygus spp.	-	Hemiptera: Miridae	Lygus plant bugs	Pest
Lymantria dispar	L.	Lepidoptera: Lymantriidae	Gypsy moth	Pest
Mangifera indica	L.	Sapindales: Anacardiaceae	mango	Tree host
Megarhyssa nortoni	Cresson	Hymenoptera: Ichneumonidae		Biological control agent; parasite
Megastigmus lawsoni	Doganlar & Hassan	Hymenoptera: Torymidae		Biological control agent; parasitoid
Megastigmus zvimendeli	Doganlar & Hassan	Hymenoptera: Torymidae		Biological control agent; parasitoid
Microctonus aethiopoides	Loan	Hymenoptera: Braconidae		Biological control agent; parasitoid
Operophtera brumata	L.	Lepidoptera: Geometridae	Winter moth	Pest

Species	Authority	Taxonomic order	Common name	Role
Ophelimus spp.	-	Hymenoptera: Eulophidae	Eucalypt gall wasp	Pest
Oryctes rhinoceros	L.	Coleoptera: Scarabaeidae	Coconut rhinoceros beetle	Pest
Oryctes rhinoceros nudivirus	-		OrNV	Biological control agent; virus, entomopathogen
Peristenus digoneutis	Loan	Hymenoptera: Braconidae		Biological control agent; parasitoid
Picea orientalis	(L.) Link	Pinales: Pinaceae	Oriental spruce	Tree host
Picea sitchensis	(Bong.) Carr.	Pinales: Pinaceae	Sitka spruce	Tree host
Pinus spp.	L.	Pinales: Pinaceae	Pine	Tree host
Profenusa canadensis	Marl.	Hymenoptera: Tenthedinidae		Pest
Profenusa thomsoni	Konow	Hymenoptera: Tenthedinidae	Ambermarked birch leaf miner	Pest
Psyllaephagus bliteus	Riek	Hymenoptera: Encyritidae		Biological control agent; parasitoid
Quadrastichus mendeli	Kim & La Salle	Hymenoptera: Eulophidae		Biological control agent; parasitoid
Quercus spp.	L.	Fagales: Fagaceae	Oak	Tree host
Rastrococcus invadens	Williams	Hemiptera: Pseudococcidae	Mango mealybug	Pest
Rhizophagus grandis	Gyllenhal	Coleoptera: Rhizophagidae		Biological control agent; predator
Rodolia cardinalis	Mulsant	Coleoptera: Coccinellidae	Vedalia beetle	Biological control agent; predator
Selitrichodes kryceri	Kim & La Salle	Hymenoptera: Eulophidae		Biological control agent; parasitoid
Selitrichodes neseri	Kelly & La Salle	Hymenoptera: Eulophidae		Biological control agent; parasitoid
Sirex noctilio	Fabricus	Hymenoptera: Siricidae	Sirex woodwasp	Pest
Sitona discoideus	Gyllenhal	Coleoptera: Curculionidae		Pest
Sitona lepidus	Gyllenhal	Coleoptera: Curculionidae	Weevil	Pest
Spondyliaspis c.f. *plicatuloides*	Froggatt	Hemiptera: Homoptera		Pest
Thaumastocoris peregrinus	Carpintero & Dellape	Heteroptera: Thaumastocoridae	Bronze bug	Pest
Torymus sinensis	Kamijo	Hymenoptera: Torymidae		Biological control agent; parasitoid

The Eucalyptus weevil,
Gonipterus *sp.*

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FAO FORESTRY PAPER

Guide to the classical biological control of insect pests in planted and natural forests

Insect pests damage millions of hectares of forest worldwide each year. Moreover, the extent of such damage is increasing as international trade grows, facilitating the spread of insect pests, and as the impacts of climate change become more evident.

Classical biological control is a well-tried, cost-effective approach to the management of invasive forest pests. It involves the importing of "natural enemies" of non-native pests from their countries of origin with the aim of establishing permanent, self-sustaining populations capable of sustainably reducing pest populations below damaging levels.

A great deal of knowledge on classical biological control has been accumulated worldwide in the last few decades. This publication, which was written by a team of experts, distils that information in a clear, concise guide aimed at helping forest-health practitioners and forest managers – especially in developing countries – to implement successful classical biological control programmes. It provides general theory and practical guidelines, explains the "why" and "how" of classical biological control in forestry, and addresses the potential risks associated with such programmes. It features 11 case studies of successful efforts worldwide to implement classical biological control.

ISBN 978-92-5-131335-0 ISSN 0258-6150

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CA3677EN/1/03.19